International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 1, 2023

Original Article

IMPORTANCE OF SUFFICIENT TIME POINTS FOR EFFICIENT PHARMACOKINETIC (PK) COMPARTMENTAL MODELING

TIRTHA NANDI*

Scientist, Clinical Pharmacology and Pharmacometrics, Alnylam Pharmaceuticals. 101 Main Street, 10th floor, Cambridge, MA 02142
Email: tirthanandi1989@gmail.com

Received: 10 Oct 2022, Revised and Accepted: 21 Nov 2022

ABSTRACT

Objective: Modeling and simulation are the two widely used terms, usually simultaneously mentioned in most PK discussions. There are several modeling strategies to model pharmacokinetic (PK) profiles. Compartmental modeling divides the body into different compartments based on the observed C-t profile and model comparison functions. Most C-t profiles are efficiently modeled using at max three compartments model (one, two, or three compartments). While there are many important applications of classical compartmental models, it emphasizes the importance of selecting the best model to explain the observed data. Therefore, initial data generation is very important. In many instances, insufficient data collection might not lead to the best model, which can be proved later costly by underpredicting or overpredicting PK parameters. This paper illustrates that adequate data collection can lead to correct model selection.

Methods: Data was generated using the three-compartmental model's explicit equation for twenty-five simulated patients with 15% random variability. Generated data were fitted to different compartmental models using sufficient time points (case a) and without enough time points (case b).

Results: In the case of a, generated data from three compartmental models was explained best by three compartmental models. In the case of b, the same data was presented better by two compartmental models. Finally, in the case of b, with sufficient time points, data generated from three compartmental models could be explained better by three compartmental models.

Conclusion: With sufficient time points, the compartmental PK model can converge to an accurate one. Although almost all pharmacometricians know the importance of time points, there is no paper with a mathematical explanation of this incident. This paper will help the current and future pharmacometricians to help design efficient *in vivo* works.

Keywords: Compartmental modeling, Pharmacokinetics (PK), Classical PK models, IV bolus, Clearance, Volume of distribution, Bioavailability

DOI: https://dx.doi.org/10.22159/ijap.2023v15i1.46553. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Pharmacokinetics (PK) is one of the major concepts in pharmaceutical sciences. Famously defined, PK is simply what the body does to the drug. PK is widely described by the acronym ADME where A stands for absorption, D stands for distribution, M stands for metabolism, and E stands for excretion (fig. 1) [1-3]. The latter two (metabolism and excretion) are summed up to be further called elimination. A drug's PK depends on numerous factors in an individual. Physicochemical factors that influence a drug's PK can include molecular weight (MW), lipophilicity (log P), solubility profile, ionization behaviors, and many more [4-8]. Even for a specific drug, its PK can differ in different individuals depending on race, gender, age, genomic and proteomic profiles, and many other factors. Less discussed, but a drug's PK can also depend on formulation factors and administration routes. For example, if a drug is administered in different ways or it is formulated as immediate release vs. controlled release, the PK of a drug can change. The major PK parameters that are used to describe a drug's behavior in an individual are volume of distribution (Vd), total clearance (CL), and bioavailability (F) [2, 3, 9]. These PK parameters are efficiently extracted from the plasma concentration versus the time profile of a drug or the C-t profile.

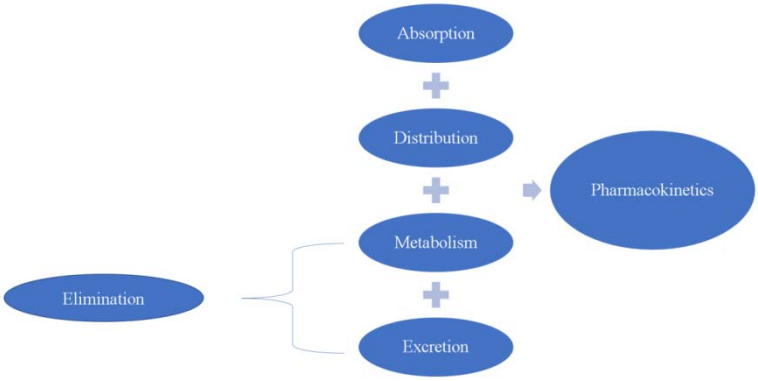

Fig. 1: Major divisions of PK

Modeling and simulation are the two widely used terms, usually simultaneously mentioned in most PK discussions [10-17]. This is because modeling PK data means looking back at the data and extracting PK parameters. In contrast, simulation helps to predict what might happen in similar cases of the drug in question, looking at future circumstances [18]. There are several modeling strategies

to model PK profiles. Compartmental modeling divides the body into different compartments based on the observed C-t profile and model comparison functions [2, 3, 9]. Most C-t profiles are efficiently modeled using at max three compartments model (one, two, or three compartments). It is noteworthy that these compartments do not necessarily have any physiological relevance. These are merely imaginary compartments used to explain the observed C-t profile. Also, within one compartment, one can practically think of many organs that are kinetically in equilibrium. For example, in one compartmental model, it is assumed that the drug distributes rapidly throughout the body and that the equilibrium is reached within minutes. Or, if the drug does not distribute at all, meaning almost all the drugs are in vascular compartments, it can also be a one-compartmental model. However, if the drug spreads to different tissues of the body and the equilibrium takes longer, there will be necessary multi-compartmental models. In multi-compartmental models, one central compartment and one or more peripheral compartments depend on the C-t profile. Peripheral compartments are reversibly connected to the main compartment. Elimination is assumed to come from the central compartment since the major eliminating organs (liver and kidney) are highly perfused and are expected to reach equilibrium relatively quickly [2, 3, 9].

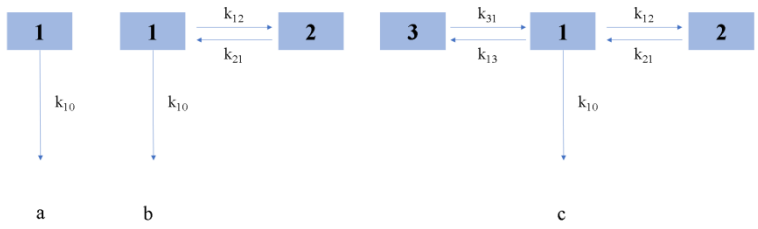

Fig. 2: a. one compartmental model, b. two compartmental models, and c. three compartmental models. Number 1 denotes central compartment, numbers 2 and 3 denote peripheral compartments. The k_{12}, k_{21}, k_{13}, and k_{31} represent inter-compartmental first-order rate constants. The k_{10} denotes irreversible first-order elimination rate constant

There are different forms of equations for different compartmental models. Simplest forms of equations are derived from the IV bolus drug administration since there is practically no absorption present in IV bolus administration. If a drug is administered by IV bolus and the drug's C-t profile is explained by one compartmental model (fig. 2a), at any time (t) drug's concentration (c_p) can be expressed by equation 1 [2, 3, 9].

$$c_p = c_0 . e^{-k_{10}t} \text{ ..... Equation 1}$$

Where c_0 is the initial drug concentration right after IV bolus drug administration.

While the equation for one compartmental model has one exponential term, if the drug follows two compartmental model (fig. 2b), it will have two exponential terms, which can be represented by equation 2 [2, 3, 9].

$$c_p = Ae^{-at} + Be^{-bt} \text{.... Equation 2}$$

Where A+B=c_0, the initial drug concentration, a and b denotes macro-rate constants formed from micro-rate constants k_{12}, k_{21}, and k_{10}.

Similarly, if the drug follows three compartmental model (fig. 2c), it will have three exponential terms, which can be represented by equation 3 [2, 3, 9].

$$c_p = Ae^{-at} + Be^{-bt} + Ce^{-ct} \text{ ..... Equation 3}$$

Where, A+B+C=c_0, the initial drug concentration, a, b, and c denotes macro-rate constants formed from micro-rate constants k_{12}, k_{21}, k_{13}, k_{31}, and k_{10}.

Compartmental models are not mechanistic models. They are mainly application-based models. And their application has made them so useful in PK. Compartmental models are relatively simpler than the widely discussed, more recent physiologically-based pharmacokinetic (PBPK) models [12, 15, 19-28], as the compartmental models need fewer mathematical inputs for successful model development. Some of the major utilities of the compartmental models include the calculation of primary PK parameters, including Vd, CL, and F, the prediction of C-t profiles for different dosage forms, including oral, dose selection to keep drug concentration within the therapeutic window, dosage regimen design for multiple doses, and the determination of pharmacokinetic variability among different populations.

While there are many important applications of classical compartmental models, it emphasizes the importance of selecting the best model that can explain the observed data. The scenario can be two-way traffic. While the data will decide which model better explains the data, at the same time, the best-fit model will decide the simulated or predicted data in the future. This is why initial data generation is very important. In many instances, insufficient data collection might not lead to the best model, and this can be proved later costly by underpredicting or overpredicting PK parameters. This paper aims to illustrate that insufficient data collection can lead to wrong model selection.

MATERIALS AND METHODS

Generation of IV dataset based on 3C model

Equation 3 was used to generate data points that follow three compartmental model. Table 1 enumerates the values used for equation 3:

Table 1: Values used for equation 3 to generate data points that follow three-compartment model

Constant in equation 3	Value (unit)
A	1000 (ng/ml)
B	700 (ng/ml)
C	10 (ng/ml)
a	2.5 ($hour^{-1}$)
b	0.5 ($hour^{-1}$)
c	0.1 ($hour^{-1}$)

Different time points ranging from 0-24 h have been used to generate simulated data. Data were generated for 25 simulated patients using equation 3 with a 15% normal function variability. Mean and standard error of the simulated data were calculated and plotted in a semi-log graph paper.

Fitting to the IV models

Sufficient time points (case a)

All the 25 simulated patients' data up to 24 h were fitted to both two-compartment and three-compartment models. One compartment was eliminated because of more than one curvature in the semi-log plot of the simulated data. Based on the Akaike Information Criterion (AIC) [29, 30] best-fit model was selected. Finally, model predicted lines were plotted with the observed data to visualize the success of each model.

Insufficient time points (case b)

The 10-hour data from all 25 simulated patients were fitted to two-compartment and three-compartment models. Due of many curvatures in the semi-log plot of the simulated data, one compartment was deleted. The best-fit model was chosen based on the Akaike Information Criterion (AIC) [29, 30]. The success of each model was then illustrated by plotting model-predicted lines against the observed data.

All the details of mathematical data analysis are provided in the supplementary files.

RESULTS

Generation of IV dataset based on 3C model

Fig. 3 shows the mean simulated data with the standard error plot in a semi-log scale.

Fitting to the IV models

Sufficient time points (case a)

Table 2 presents the model comparison function for case a, where sufficient time points were considered. Since the three-compartmental model produced a lower AICc value, it was considered the better model to explain the data. Fig. 4 shows individual model fittings. Finally, table 3 shows all the micro-rate constants and volume of central compartment obtained from both model fittings.

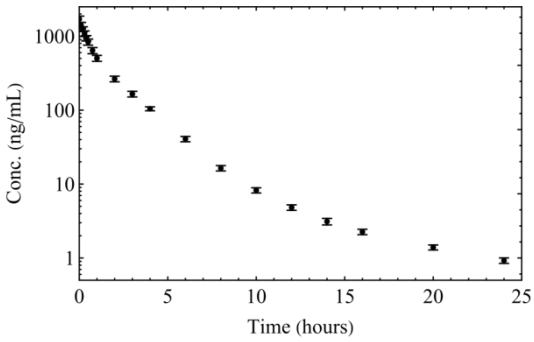

Fig. 3: Semi-logarithmic plot of mean C-t profile with standard error of 25 simulated patients

Table 2: Model comparison functions for case a

Function	Two compartmental model	Three compartmental model
AICc	132.367	114.392
R^2	0.9992	0.9998

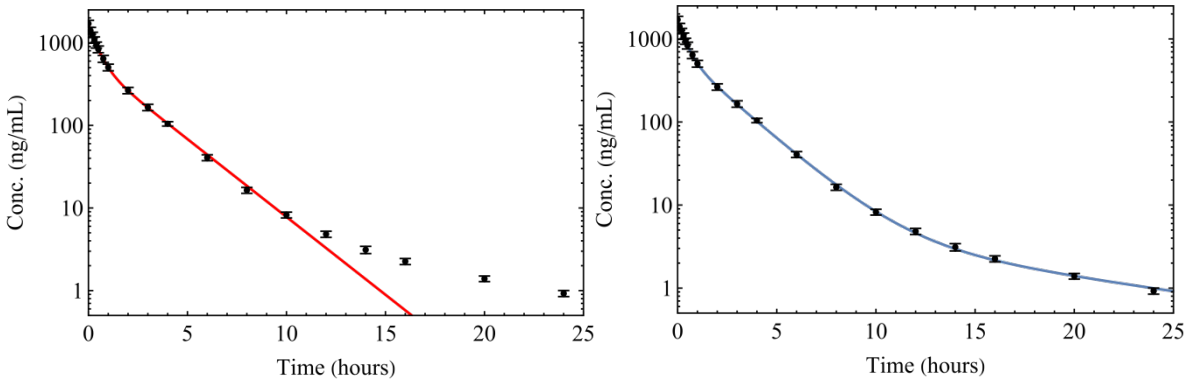

Fig. 4: a. Two compartmental model fitting for case a, b. Three compartmental model fitting for case a

Table 3: Micro-rate constants and volume of central compartment for both model fittings in case a. Data shows as mean (SE.)

Parameter	Two compartmental model	Three compartmental model
k12	0.6890 (0.0416)	0.7172 (0.0311)
k21	1.0717 (0.0554)	1.2555 (0.0783)
k13	NA	0.0305 (0.0049)
k31	NA	0.0826 (0.0383)
k10	0.9024 (0.0155)	0.8939 (0.0091)
Vc	5.9537 (0.0722)	5.9289 (0.0394)

Insufficient time points (case b)

Table 4 presents the model comparison function for case b where sufficient time points were not considered (just 10 h). Since the two compartmental models produced lower AICc values, it was considered the better model to explain the data. Fig. 5 shows individual model fittings. Finally, table 5 shows all the micro-rate constants and volume of the central compartment obtained from both model fittings.

Table 4: Model comparison functions for case b

Function	Two compartmental model	Three compartmental model
AICc	106.676	121.484
R^2	0.9998	0.9998

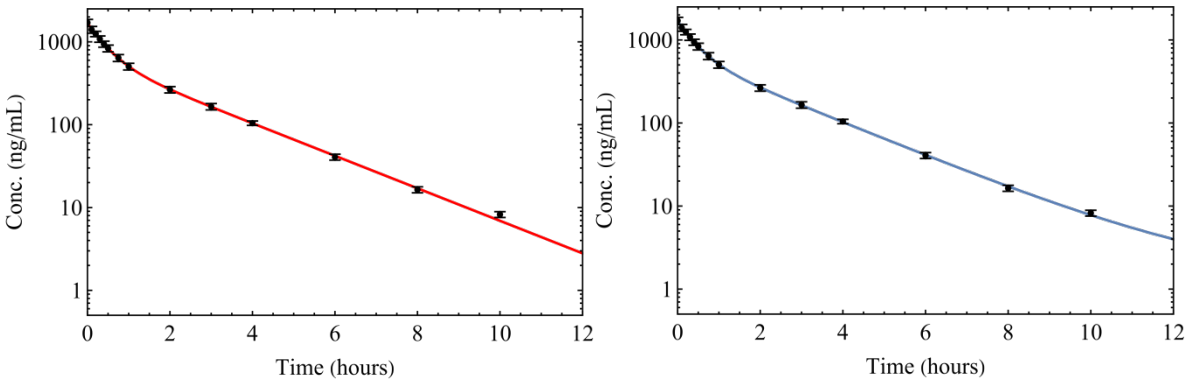

Fig. 5: a. Two compartmental model fitting for case b, b. Three compartmental model fitting for case b

Table 5: Micro-rate constants and volume of central compartment for both model fittings in case a. Data shows as mean (SE.)

Parameter	Two compartmental model	Three compartmental model
k12	0.7094 (0.0264)	0.7156 (0.0486)
k21	1.1533 (0.0315)	1.2163 (0.1145)
k13	NA	0.0263 (0.2828)
k31	NA	0.0455 (0.7377)
k10	0.9081 (0.0102)	0.8912 (0.2999)
Vc	5.9403 (0.0439)	5.9326 (0.0495)

DISCUSSION

When a drug is available in systemic circulation, it needs time to equilibrate throughout the body. Within this time, drugs reversibly distribute to different tissues of the body depending upon the blood flow to the organ and the apparent affinity of the drug to that particular tissue [2, 3, 9]. Some of the body's organs are highly perfused, and the equilibrium to those organs is relatively quick, including the liver and kidney. These organs are therefore considered in the central compartment in a compartmental modeling approach. Other tissues with less blood perfusion or deeper anatomical position will be considered in the peripheral tissues. Drugs' physicochemical properties can also dictate which organ might be in the central compartment versus the peripheral compartment. For example, for a highly lipophilic drug, the brain can be considered in a central compartment since the drug can cross the blood-brain barrier. Conversely, the brain will merge in the peripheral compartment for less lipophilic drugs due to higher equilibration time.

All these physiologic plus physicochemical properties can determine the time to equilibrate the drug inside the body. For a rapid IV bolus administration, this feature is more visible. The drug's time to equilibrate inside the body is called the distributive phase. After the distributive phase, equilibration is reached, and the phase is called the elimination phase. Although distribution and elimination occur simultaneously in the distributive phase, only elimination is apparent in the elimination phase. This is why the drug concentration in the plasma drops faster in the distributive phase than in the elimination phase. The presence of a distributive phase will depend on how frequently blood samples are taken. Multi-compartment PK models are created based on the degree of post-distribution equilibrium and the number of tissues with similar kinetic properties.

Inaccurate estimations of the elimination phase of the IV data might result in incorrect calculations of the intercompartmental and elimination rate constants. For instance, if a drug has three compartmental characteristics but the IV bolus PK data only shows two compartmental characteristics due to shorter blood collection time points or the analytical sensitivity limitation, it may significantly overpredict the elimination rate constant, causing the oral PK profile to degrade rapidly. Therefore, scientists must draw blood and samples analyzed over an extended period to properly ascertain the IV PK profile's tail end for the accurate characterization of the disposition function utilizing conventional compartmental PK models. Given that the sensitivity of LC-MS/MS will also catch the post-distributive phase, blood collection up to 5 half-lives may typically provide sufficient blood collection [27, 31-34].

This research aimed to evaluate the sufficiency of time points for successful pharmacokinetic compartmental modeling. Initially, the data was generated using a three-compartmental model. For this purpose, the explicit equation for three compartmental pharmacokinetic models was used using random numbers. A random error of 15% was applied to generate data for twenty-five simulated patients. This random error was added while generating simulated patients' data to consider experimental variability and patient-to-patient inter-individual differences. However, the data variability might be higher in practice, which is typical of *in vivo* experiments [33, 35-40].

The next job was to determine if the PK modeling could converge to three compartmental models better than other models statistically. Furthermore, since the data was generated using a three-compartmental model, it was aimed to see if it is still the best model to explain its data. For this purpose, two cases were designed. One in which sufficient time points were considered (case a) and the other in which sufficient time points were not considered (case b).

Before starting to model PK data, the first step is to look at the data. In a semi-logarithmic plot, the number of an apparently linear region can suggest which model might be followed by the generated data. In the case of a, more than two linear regions were apparent from the simulated data. Also, the model comparison functions suggested that the three-compartmental model better explained the data. On the other hand, in the case of b, only two linear regions were apparent from the simulated data. Also, the model comparison functions suggested that the two-compartmental model better explained the data. From these two cases, data generation up to sufficient time points is a prerequisite for successful compartmental PK modeling.

Now the next question becomes, how long time points are sufficient? It depends on several factors. One of the limiting factors might be analytical sensitivity. With very sensitive analytical techniques, it is practically possible to characterize the terminal portion of the C-t profile. With the advent of newer technologies, picomolar

concentrations are also possible to determine with scientific confidence. Therefore, this research recommends blood collection until it reaches the lower limit of quantitation of the analytical instrument. Another thing to consider might be the dose for the compartmental PK modeling. Within linear PK, using a higher dose might help to better characterize the terminal phase of the C-t profile without compromising the analytical sensitivity.

CONCLUSION

Compartmental PK modeling is still one of the most useful modeling techniques in PK. However, without proper characterization or improper modeling might lead to inaccurate PK parameters. Sufficient time points are one of the simplest way to avoid error in compartmental PK modeling.

ACKNOWLEDGEMENT

This research is highly acknowledging Dr. Zeyuan Wang, West Point, Merck for his help in teaching Mathematica.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Alavijeh MS, Palmer AM. The pivotal role of drug metabolism and pharmacokinetics in the discovery and development of new medicines. Drugs. 2004;7(8):755-63. PMID 15334309.
2. Gibaldi M, Perrier D. Pharmacokinetics Swarbrick J. editor. New York: Marcel Dekkar, Inc; 1982. p. 494.
3. Hedaya MA. Basic pharmacokinetics. CRC Press; 2012.
4. Dokoumetzidis A, Kalantzi L, Fotaki N. Predictive models for oral drug absorption: from *in silico* methods to integrated dynamical models. Expert Opin Drug Metab Toxicol. 2007;3(4):491-505. doi: 10.1517/17425225.3.4.491, PMID 17696801.
5. Huang W, Lee SL, Yu LX. Mechanistic approaches to predicting oral drug absorption. AAPS J. 2009;11(2):217-24. doi: 10.1208/s12248-009-9098-z, PMID 19381841.
6. Yu LX, Amidon GL. A compartmental absorption and transit model for estimating oral drug absorption. Int J Pharm. 1999;186(2):119-25. doi: 10.1016/s0378-5173(99)00147-7, PMID 10486429.
7. Lin L, Wong H. Predicting oral drug absorption: mini review on physiologically-based pharmacokinetic models. Pharmaceutics. 2017;9(4):41. doi: 10.3390/pharmaceutics9040041, PMID 28954416.
8. Löbenberg R, Kramer J, Shah VP, Amidon GL, Dressman JB. Dissolution testing as a prognostic tool for oral drug absorption: dissolution behavior of glibenclamide. Pharm Res. 2000;17(4):439-44. doi: 10.1023/a:1007529020774, PMID 10870988.
9. Biopharmaceutics SL. Encyclopedia of pharmaceutical science and technology. 4th ed. CRC Press; 2013. p. 195-210.
10. Bonate PL. Pharmacokinetic-pharmacodynamic modeling and simulation. Springer; 2011.
11. Kim TH, Shin S, Shin BS. Model-based drug development: application of modeling and simulation in drug development. J Pharm Investig. 2018;48(4):431-41. doi: 10.1007/s40005-017-0371-3.
12. Rowland M, Lesko LJ, Rostami Hodjegan A. Physiologically based pharmacokinetics is impacting drug development and regulatory decision making. CPT Pharmacometrics Syst Pharmacol. 2015;4(6):313-5. doi: 10.1002/psp4.52, PMID 26225258, PMCID PMC4505824.
13. Zhao P, Zhang L, Grillo JA, Liu Q, Bullock JM, Moon YJ. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. Clin Pharmacol Ther. 2011;89(2):259-67. doi: 10.1038/clpt.2010.298, PMID 21191381.
14. Zhao P, Rowland M, Huang SM. Best practice in the use of physiologically based pharmacokinetic modeling and simulation to address clinical pharmacology regulatory questions. Clin Pharmacol Ther. 2012;92(1):17-20. doi: 10.1038/clpt.2012.68, PMID 22713733.
15. Zhuang X, Lu C. PBPK modeling and simulation in drug research and development. Acta Pharm Sin B. 2016;6(5):430-40. doi: 10.1016/j.apsb.2016.04.004. PMID 27909650, PMCID PMC5125732.
16. Dave VS, Patel MB. Method development and validation of difference UV spectrophotometric estimation of lafutidine in bulk and pharmaceutical dosage form. Int J Pharm Pharm Sci. 2012;4(4):284-7.
17. Sornsuvit C, Niamhun N, Luengpiansamut N, Sangsrijan S, Niwatananum W, Kaewvichit S. Pharmacokinetics and bioequivalence studies of warfarin sodium 5 milligrams tablet in healthy Thai subjects. Int J Pharm Pharm Sci. 2015;7(3):219-22.
18. Iswandana R, Aisyah P, Syahdi RR. Prediction analysis of pharmacokinetic parameters of several oral systemic drugs using *in silico* method. Int J App Pharm. 2020;12(1):260-3. doi: 10.22159/ijap.2020.v12s1.FF057.
19. Bruyere A, Decleves X, Bouzom F, Ball K, Marques C, Treton X. Effect of variations in the amounts of P-glycoprotein (ABCB1), BCRP (ABCG2) and CYP3A4 along the human small intestine on PBPK models for predicting intestinal first pass. Mol Pharm. 2010;7(5):1596-607. doi: 10.1021/mp100015x, PMID 20604570.
20. Ferl GZ, Theil FP, Wong H. Physiologically based pharmacokinetic models of small molecules and therapeutic antibodies: a mini-review on fundamental concepts and applications. Biopharm Drug Dispos. 2016;37(2):75-92. doi: 10.1002/bdd.1994, PMID 26461173.
21. Garg A, Balthasar JP. Physiologically-based pharmacokinetic (PBPK) model to predict IgG tissue kinetics in wild-type and FcRn-knockout mice. J Pharmacokinet Pharmacodyn. 2007;34(5):687-709. doi: 10.1007/s10928-007-9065-1, PMID 17636457.
22. Kuepfer L, Niederalt C, Wendl T, Schlender JF, Willmann S, Lippert J. Applied concepts in PBPK modeling: how to build a PBPK/PD model. CPT Pharmacometrics Syst Pharmacol. 2016;5(10):516-31. doi: 10.1002/psp4.12134, PMID 27653238, PMCID PMC5080648.
23. Li J, Wu J, Bao X, Honea N, Xie Y, Kim S. Quantitative and mechanistic understanding of AZD1775 penetration across human blood–brain barrier in glioblastoma patients using an IVIVE–PBPK modeling approach. Clin Cancer Res. 2017;23(24):7454-66. doi: 10.1158/1078-0432.CCR-17-0983, PMID 28928160.
24. Offman E, Edginton AN. A PBPK workflow for first-in-human dose selection of a subcutaneously administered pegylated peptide. J Pharmacokinet Pharmacodyn. 2015;42(2):135-50. doi: 10.1007/s10928-015-9406-4, PMID 25650156.
25. Sager JE, Yu J, Ragueneau Majlessi I, Isoherranen N. Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. Drug Metab Dispos. 2015;43(11):1823-37. doi: 10.1124/dmd.115.065920, PMID 26296709, PMCID PMC4613950.
26. Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang SM. Application of physiologically based pharmacokinetic (PBPK) modeling to support dose selection: report of an FDA public workshop on PBPK. CPT Pharmacometrics Syst Pharmacol. 2015;4(4):226-30. doi: 10.1002/psp4.33, PMID 26225246.
27. Wong H, Chow TW. Physiologically based pharmacokinetic modeling of therapeutic proteins. J Pharm Sci. 2017;106(9):2270-5. doi: 10.1016/j.xphs.2017.03.038. PMID 28392453.
28. Zhao P, Zhang L, Grillo JA, Liu Q, Bullock JM, Moon YJ. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. Clin Pharmacol Ther. 2011;89(2):259-67. doi: 10.1038/clpt.2010.298, PMID 21191381.
29. Sakamoto Y, Ishiguro M, Kitagawa G. Akaike information criterion statistics. Dordrecht, The Netherlands: D Reidel; 1986. p. 81.

30. Yamaoka K, Nakagawa T, Uno T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharm. 1978;6(2):165-75. doi: 10.1007/BF01117450, PMID 671222.
31. Erratum: blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2017;8(3):153. doi: 10.4103/0976-500X.215702, PMID 29081629.
32. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol. 2001;21(1):15-23. doi: 10.1002/jat.727, PMID 11180276.
33. Lundell A, Bergqvist D, Mattsson E, Nilsson B. Volume blood flow measurements with a transit time flowmeter: an *in vivo* and *in vitro* variability and validation study. Clin Physiol. 1993;13(5):547-57. doi: 10.1111/j.1475-097x.1993.tb00470.x, PMID 8222539.
34. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010;1(2):87-93. doi: 10.4103/0976-500X.72350, PMID 21350616.
35. Chawla G, Bansal A. A means to address regional variability in intestinal drug absorption. Pharm Technol. 2003;27(2):50-68.
36. Dimitrov S, Detroyer A, Piroird C, Gomes C, Eilstein J, Pauloin T. Accounting for data variability, a key factor in *in vivo*/*in vitro* relationships: application to the skin sensitization potency (*in vivo* LLNA versus *in vitro* DPRA) example. J Appl Toxicol. 2016;36(12):1568-78. doi: 10.1002/jat.3318, PMID 27080242.
37. Howgate EM, Rowland Yeo K, Proctor NJ, Tucker GT, Rostami Hodjegan A. Prediction of *in vivo* drug clearance from *in vitro* data. I: Impact of inter-individual variability. Xenobiotica. 2006;36(6):473-97. doi: 10.1080/00498250600683197, PMID 16769646.
38. Pham LL, Watford SM, Pradeep P, Martin MT, Thomas RS, Judson RS. Variability *in vivo* studies: defining the upper limit of performance for predictions of systemic effect levels. Comp Toxicol. 2020;15:100126. doi: 10.1016/j.comtox.2020.100126.
39. Vinarov Z, Abdallah M, Agundez JAG, Allegaert K, Basit AW, Braeckmans M. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review. Eur J Pharm Sci. 2021;162:105812. doi: 10.1016/j.ejps.2021.105812, PMID 33753215.
40. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev. 2009;41(2):89-295. doi: 10.1080/03602530902843483, PMID 19514967.