10.1 Pharmacokinetic Modeling

Drug research encompasses several disciplines united by a common goal, namely the development of novel therapeutic agents. However, the research for new drug can be divided functionally into two stages, viz. discovery and development. The process of drug development consists of setting up a working hypothesis of the target enzymes or receptors for a particular disease, establishing suitable mathematical models or surrogate markers to be biological activities and screening the new drug molecules for in vitro or in vivo biological activities (Ahmed et al. 2012; Aronow et al. 2000; Basavarajaiah 2018). Overwhelmingly, the drug development process will be too many critics, efforts concealed to focus on evaluation of the toxicity and efficacy of new drug candidates. The mathematical model to know the characteristics of time of drug absorption, metabolism and excretions and relationship of these processes to the intensity and time course of the therapeutic and toxicology effects of drug (Boonleang et al. 2007; Beringer et al. 2005; Breiman 2001). The PK model describes the process whereby specific mode and by a specific dose is handled by the body leading to the specific drug concentration in different tissues or organs. Further, the part of drug will reach the sites of action and excretes into pharmacodynamic action. The PK is used in the clinical settings to enhance safe and effective therapeutic management of individual patients or subjects. This application is termed as clinical pharmacokinetic. The salient principles of PK model are presented as follows.

1. Design and development of new drugs for improving therapeutic effectiveness
2. Design and development of an optimum formulation for better use of the drug
3. Design and development of controlled or targeted release formation
4. Select the appropriate route for drug administration
5. Select the right drug for particular illness
6. Predict and explain drug—food and drug-to-drug interaction
7. Design an appropriate multiple dosage regimen
8. Therapeutic drug monitoring in individual patients
9. Dosage adjustment in situation of altered physiology and drug interaction (Fig. 10.1)
It is a process in which the unchanged proceeds from site of administration to the site of measurement within the body.

The transfer of drug from blood to the extra vascular fluids and tissues. It is a rapid and reversible process.

It is the biochemical conversion of drug into another chemical forms.

It is irreversible loss of drug from the site of measurement.

It is the irreversible loss of chemically unchanged drug by various routes. This can occur through urine, biliary secretion, saliva, sweat, milk and respiratory route.

Hard drug: non metabolism drugs are called hard drug, drugs excreted primarily through either bile or kidney. Eg Biophosphate, lisinopril.

Soft drug: it is pharmacologically active it undergoes a predictable and controllable metabolism to non toxic and inactive metabolites. Eg. Remifentanil.

**Fig. 10.1** Flowchart of ADME process in human body.
10.2 Application of PK Mathematical Model in New Drug Development Process

To understand the process of absorption, distribution, elimination of drug, which affects onsets and intensity of biological response

To access plasma drug concentration response to given dose which is considered as more appropriate parameter than intrinsic PK activity

In design and utilization of in vivo model that can evaluate dissolution characteristics of new compound formulated as new drug formulation and establish meaningful IVIVC

In design and development of new drug and their appropriate dosage regimen

In safe and effective management of patients by improving drug therapy

To understand the concept of bioavailability which has been used to evaluate and monitor in vivo performance of new dosage forms and genetic formulation

To carry out the bioavailability and bioequivalence tests

We can use the PK principles in the development of the various new drug development processes (Fig. 10.2).

Dosing of drug in obese patients

Ideal body weight (IBW) is calculated as follows:

\[
\text{IBW (Men)} = 50 \text{ kg} \pm 1 \text{ kg per 2.5 cm above or below 150 cm in height}
\]

\[
\text{IBW (women)} = 45 \text{ kg} \pm 1 \text{ kg per 2.5 cm above or below 150 cm in height}
\]

Fig. 10.2 Approaches for dosage regimen in new drug
10.3 Pharmacokinetic (PK) Mathematical Model

PK model is a hypothetical structure used to describe the fate of a drug in a biological system following administration. All PK models embedded or tailored with some mathematical simulation from original database of drug information, so many attributes (clinical) and serological markers and candidates were mathematically simulated in the interest. The PK models it means of expressing mathematically or quantitatively, time course of drug throughout the body and compute meaningful PK parameters (Chang et al. 2015; Caro et al. 2012). It is thought of numerical figures which express in the form of mathematical form or equations to solve the real-world problems of drug development process. The PK model elaborated on the basis of known anatomy and physiology of human and other animals to characterize the behavior of drug in patients, predicting one of drug in various fluids with dosage regimen, calculating optimum drug regimen for individual patients, evaluating bioequivalence between different formulation, and also drug-to-drug interaction and drug-to-food interaction are also explained in a specified period of time (Ceckova-Novotna et al. 2006) (Fig. 10.3).

There are three main reasons due to which the data is subjected to modeling, viz. descriptive to describing the drug kinetics in a simple way, to predict the time course of the drug after multiple dosing based on single dose data, and we estimate the absorption capacity and profile of drug from iv database and model which will help us explain the unclear observations and statements from all piece of quantitative drug information (Cox 1990). The compartment model is not a real physiological or automatic region, but an imaginary or hypothetical one consists of tissues or group of tissues with similar blood flow and affinity which can help in the visual representation of various rate processes involved in drug disposition. The model has been classified into central and peripheral compartments: central model escalates blood and highly perfused tissues such as heart, kidney, lungs, and liver, whereas peripheral model can accelerate poorly perfused tissues such as fat and bone (Figs. 10.4 and 10.5).

The rate of change of plasma concentration versus time was modeled in the following manner:

\[ R = \frac{C_p}{t} = K_e * C_p, \]  \hspace{1cm} (10.1)

![Fig. 10.3 Model types in clinical research](image-url)
\[
\frac{dx_0}{dt_0}, \frac{dx_1}{dt_1}, \frac{dx_2}{dt_2}, \ldots, \frac{dx_n}{dt_n} = \text{rate in availability} - \text{rate out (elimination)}.
\]

---

**Drug product** --- \[\rightarrow\] **Drug in blood** --- \[\rightarrow\] **Excretion**

- Tissue
- Tissue and receptive sites
- Metabolism

---

**Fig. 10.4** Variation of plasma value with respect to \(P\) values (\(P\) value)

**Fig. 10.5** PK model absorption and elimination phase (\(P\) value)
If rate out or elimination follows first order kinetics is

\[
\frac{dx}{dt} = -ke^{(a+bx)^t},
\]

(10.2)
where \(a\) is the intercept of the slope or induction level of absorption and \(k\) is proportionally constant and \(b\) is the change in time and plasma concentration, \(x\) can take values of plasma concentration with respect to different time period.

Integrating Eq. (10.2), the given eqn becomes

\[
\ln x = \ln X_0 - k \left[ \ln a + b \ln xi \right] t.
\]

\(X_0 = \) Amount of drug injected at time “\(t\)”, \(\, t = \) zero, i.e., initial or inception of drug

\[
X = X_0 e^{-k(a+bx)^t},
\]

(10.3)

\[
\log X = \log X_0 - \frac{k(a+bx)}{2.303},
\]

(10.4)

Since it is difficult to directly determine the amount of drug in body and we use relationship that “\(C\)” exists between drug concentration. In plasma “\(C\)” and “\(X\)” thus eqn becomes

\[
X = V_d C,
\]

(10.5)

\[
\log C = \log C_0 - \frac{k(a+bx)}{2.303}.
\]

(10.6)

Equation (10.6) elimination of half-life

\[
T_{1/2} = \frac{0.693}{Ke(a+bx)^t},
\]

(10.7)

\[V_d = \frac{\text{Amount of drug in body}}{\text{Clt}} = \frac{X}{C}, \text{ e.g., } 30 \text{ mg} \]

IV bolus, plasma concentration = 0.732 mg/mL.

\[V_d = 30 \text{ mg}/0.732 \text{ mcg/mL} = 30,000 \text{ mcg} = 41 \text{ L} \]

For drug given as IV bolus

\[Vd(\text{area}) = \frac{x_0}{k e^{(a+bx)}} \ast \text{AUC}. \]

(10.8)

For drug administered extra Vas

\[Vd(\text{area}) = \frac{fx_0}{k e^{(a+bx)}} \ast \text{AUC}. \]

(10.9)

Clearance = rate of elimination/plasma drug concentration

\[cl = \frac{dx}{t}. \]

Renal clearance = \(\left(\frac{\text{Rate of elimination by kidney}}{C}\right)\)

Hepatic clearance = \(\left(\frac{\text{Rate of elimination by Liver}}{C}\right)\)

Other organ = \(\left(\frac{\text{Rate of elimination by organ}}{C}\right)\)

Total body clearance was calculated from the following eqn:

\[\text{CIT} = cLR + Clh + ClOthers, \]

(10.10)

\[\text{CIT} = k e^{-(a+bx)} \ast Vd. \]

(10.11)

The effective clearance of drug was smoothened into accurate and non-accurate clearance, it was estimated from the binary logistic regression, the accurate will be coded as 1, and non-accurate will be coded as 0. Perform the logistic regression with two or more dependent variables. The equation will give rise to the total clearance of the administered drug (Cusack et al. 1979; Chandrasekhar 1943).
\[ \text{CIT (odd)} = k \times \frac{\exp(d)}{1 + \exp(d)} \times V_d \]

where
\[ \exp(d) = e^{(-a + bx)^{\gamma}}, \quad (10.12) \]

\[ \text{CIT (odd)} = k \times \ln\left(\frac{\text{odd}}{1 - \text{odd}}\right) \times V_d, \quad (10.13) \]

or
\[ \text{CIT (odd)} = k \times \ln\left(\frac{p}{1 - p}\right) \times V_d \]

where “P” is the probability values with respect to plasma concentration and time (hours) (Fig. 10.6).

\[ \text{CIT (odd)} = k \times \ln\left(\frac{0.35}{1 - 0.35}\right) \times 41L \]

\[ k = \text{age constant} \]

\[ \text{CIT} = 25 \times \ln\left(\frac{0.35}{1 - 0.35}\right) \times 41L \]

\[ \text{CIT} = 631.54 \times \text{accurate clearance was done} \]

10.4 Area Under Curve (AUC) (mcg/mL)

AUC is a measure of the total systematic exposure of a drug. Thus it is calculated from concentration time data. AUC is not a primary PK parameter, it is derived from CL and dose

\[ \text{AUC} = \int_{0}^{\infty} C(t) \, dt = \int_{0}^{\infty} \frac{D}{V} e^{\left(-\frac{cT}{V}\right)} \, dt, \]

\[ \text{AUC} = \frac{D}{V} \times \frac{1}{CL} = \frac{D}{CL}, \quad (10.14) \]

where “D” is the level of distribution with respect to bioavailability, and V is the volume (Fig. 10.7).

Fig. 10.6 Logistic regression plot with varying plasma drug concentration and time
Volume distribution is defined as the volume that accommodates all drugs in the body, if concentration was the same as in plasma, the eqn becomes

\[ V = \frac{\text{Dose administered IV}}{\text{Plasma concentration}}, \] e.g., total drug in the body 1000 mg, and plasma concentration is 50 mg/mL

The volume equals \( \frac{1000}{50} = 20 \text{L} \)

\[ \text{AUC}_{2-3} = \frac{CP^2 + CP^3}{2} \left( t^3 - t^2 \right), \quad (10.15) \]

\[ \text{AUC}_{2-3} = \left\{ \frac{CP^3 + CP^2}{2} \left( t^2 - t^1 \right) \right\} + \frac{CP^3 + CP^2}{2} \left( t^3 - t^2 \right) + \cdots + \frac{CP^3 + CP^2}{2} \left( t^n - t^{n-1} \right), \quad (10.16) \]

\[ \text{AUC} = \sum_{i=1}^{n} \frac{t^{i+1} - t^i}{2F_i} \cdot C_j + C_t \cdot tC_{j+1}. \]

Total area under the first moment curve

\[ F = \frac{\text{AUC}_{\text{Oral}}}{\text{AUC}_{\text{IV}}} \times \frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{Oral}}}, \]

\[ F_r = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{std}}} \times \frac{\text{AUC}_{\text{std}}}{\text{AUC}_{\text{test}}}. \]

\( T_{\text{max}} \) is the time taken for drug to reach peak concentration in plasma called as the time of peak concentration. Particularly important in assessing the efficacy of the drug used to treat
acute conditions like malignancy, pain and HIV-infected cases (Delafuente et al. 2008; El Desoky 2007; EMEA 2006).

Half-life: The half-life will depend on the time taken for the drug concentration or amount in the body to fall by one-half such as CP = 1/2CP0 (Fig. 10.8).

\[ t_{1/2} = \frac{0.693}{\text{K}_{el}} \]

\[ \text{K}_{el} \text{(elimination rate constant)} = \frac{-dCp/dt}{Cp} \]

\[ \text{K}_{el} = \frac{\ln(Cp_1) + \ln(Cp_2)}{t_2 - t_1} \]

---

**Example of Kel**

| Drug           | Kel (1/h) |
|----------------|-----------|
| Acetaminophen  | 0.28      |
| Diazepam       | 0.021     |

---

**Drug** | **Kel (1/h)**
---|---
Digoxin | 0.017
Gentamycin | 0.35
Lidocaine | 0.43
Theophylline | 0.063

---

\[ \text{Total AUC}_{0-\infty} = \left( \frac{Cp_0 + Cp_1}{2} t_1 + \frac{Cp_1 + Cp_2}{2} t_2 - t_1 \right) + \frac{Cp_2 + Cp_3}{2} t_3 - t_2 \ldots \frac{Cp_{last}}{K_{el}} \]

---

**Fig. 10.8** Determination of ROC by regression method

**Regression Line** (y = -5.87X + 90.06)
Model = $-5.8693x + 90.05 \ (R^2 = 0.98)$

$SSX = 214.30; \ SP = -1257 - 84; \ b = SP/SSX = -1257.85/214.31 = -5.86$

$a = M_y - bM_x = 47.62 - (-5.87 * 7.23) = 90.05$

$$t_{1/2} = \frac{0.693}{kel} \times \frac{1}{Y}$$

$Cp = 71, \ kel = 0.34$

$\hat{y} = -5.87X + 90.06$

Substitute “$X$” value for the above model (when $X = 2$ h), the estimated value of

$$\hat{y} = -5.87(2) + 90.06,$$

$$\hat{y} = -11.74 + 90.06,$$

$$\hat{y} = 78.32,$$

$$t_{1/2} = \frac{0.693}{kel} \times 78.32 \ (substitute \ kel = 0.34),$$

$$t_{1/2} = \frac{0.693}{0.34} \times 78.32,$$

$$t_{1/2} = 2.03 \times 78.32 = 159.6. \ \ (10.17)$$

Equation (10.17) determines that the half-life of the drug is 159.60 times observed in the human body

$$CP = CP_0 \times e^{-kel\nu},$$

$$CP_0 = \frac{CP}{e},$$

where $CP = 71, \ kel = 0.34$,

$$CP_0 = \frac{71}{0.34} = 100, \ \ (10.18)$$

$$AUC_{0-1} = \frac{100 + 71}{2} \times 1 = 85.50,$$

$$AUC_{Last-c} = \frac{CP_{Last}}{kel} \times \frac{5}{0.34} = 14.70 \ mg.h / L$$

(value of 5 was obtained from the illustration) (Fig. 10.9).

Step II: Integration method

$$AUC = \int_{0}^{3} X^2 dx,$$

$$= \left[ \frac{X^{n+1}}{n+1} \right]_{1}^{4}$$

**Fig. 10.9** Steps for determination of AUC
where \( X = 80, \ln 80 = 4.38, \)

\[
\frac{X^2}{2} \left[ \frac{4.38^2}{2} \right] = \left[ \frac{4.38^2}{2} \right] \cdot 100,
\]

\[
\text{AUC} = 33.43
\]

\[
y(AUC) = \int_a^b f(x) dx,
\]

\[
y(AUC) = f(b) - f(a).
\]

The AUC was tested by Gaussian distribution:

\[
Z_{AUC} = \frac{\bar{x}_i - C_{\text{max}}}{\sigma_i},
\]

where \( C_{\text{max}} = \mu \) population means

\[
\sigma_i = \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x})^2,
\]

\( \bar{x}_i = \) sample mean of plasma concentration \( i \)th drug,

\( \sigma_i = \) Sample SD of \( i \)th drug,

\( Z_{AUC} \) Tested by normal distribution curve and refer the table value and reject the \( H_0 \).

10.5 Receiver Operating Characteristics Analysis for Fitting AUC

The ROC curve is plotting of values of false positive rate (FPR) versus the true positive rate (TPR) for all possible values ranged from 0 to 1. The higher the ROC curve better the fit. In fact, the AUC can be used for this purpose. The closer is to 1 (maximum value) the better the fit. Values close to 0.50 show that the model’s ability to discriminate between success and failure is due to chance (Gandhi et al. 2004; Jambhekar and Breen 2009; Khorsan and Crawford 2014). Typically used to evaluate the performance of drug in human body, AUC for plasma concentration, performance of machine learning language for identification of new algorithms, identification of rare gene for malignancy, specificity, and sensitivity of the drug, etc.

\[
\text{TPR} = \frac{TP}{TP + FN},
\]

\[
\text{FPR} = \frac{FP}{FP + TN}.
\]

TPR and FPR are plotted for different threshold value; threshold values may often be chosen based on the optimum point in the ROC curve. Thumb rule of ROC is the value lies between 0.90 and 1; excellent, 0.80–0.90 good; 0.70–0.80 fair; 0.60–0.70 poor and values lie between 0.50 and 0.60 are fail.

Illustration: The sensitivity and specificity of Efavirenz fixed-dose regimen efficacy were obtained from RCT double blind, and the researcher wishes to extrapolate the sensitivity and specificity by using receiver operating characteristic analysis.

Model formulation: The collected data sets were categorized based on the rating of efficacy, 90–100% coded as 5; excellent, 80–90% coded as 2; 70–80% coded as 3 and 60–70% coded as 2 and 50–60% coded as 1 (\( n = 50 \) cases) (Fig. 10.10).

1. Number of Cases: 50
   Number Correct: 42
   Accuracy: 84%
   Sensitivity: 88%
   Specificity: 80%; Positive Cases Missed: 3;
   Negative Cases Missed: 5
   (A rating of 3 or greater is considered positive.)
   Fitted ROC area: 0.905
   Empiric ROC area: 0.892
   Summary of ROC curve:
   Area = 0.9055 ± 0.042

2. Initial values of parameters
   \( A = 1.7621 \)
   \( B = 0.9538 \)
   \( Z(K) = -0.1507 \quad 0.8415 \quad 1.4053 \quad 1.7511 \)
   \( \log l = -68.6817 \)
3. Final values of parameters
   Procedure converges after six iterations.
   \[ A = 1.8683 \]
   \[ B = 1.0118 \]
   \[ Z(K): -0.1202 \ 0.7566 \ 1.2524 \ 2.0602 \]
   \[ \log L = -65.0240 \]

4. Variance–covariance matrix
   \[ A \begin{pmatrix}
   0.2517 & 0.1303 & 0.0546 & 0.0592 & 0.0391 \\
   0.0306 & 0.1303 & 0.1384 & 0.0205 & -0.0005 \\
   -0.1332 & -0.0005 & 0.0546 & 0.0205 & 0.0363 \\
   0.0546 & 0.1384 & 0.0205 & 0.0626 & 0.0633 \\
   0.0592 & 0.0205 & 0.0626 & 0.0363 & 0.0274 \\
   \end{pmatrix} \]

5. Correlation matrix (Table 10.1)
   \[ A \begin{pmatrix}
   1.0000 & 0.6983 & 0.4350 & 0.4480 & 0.2526 \\
   -0.1225 & 0.6983 & 0.2207 & -0.0049 & -0.3270 \\
   -0.7182 & 0.2207 & 1.0000 & 0.5507 & 0.3551 \\
   0.1018 & -0.0049 & 0.5507 & 1.0000 & 0.7492 \\
   0.4414 & \end{pmatrix} \]

---

### Table 10.1
Estimated binormal ROC curve with asymmetric 95% confidence interval

| FPF  | TPF  | CI-95%       |
|------|------|--------------|
| 0.005| 0.2301| [0.0169,0.7407]|
| 0.010| 0.3135| [0.0430,0.7718]|
| 0.020| 0.4168| [0.0996,0.8061]|
| 0.030| 0.4860| [0.1545,0.8282]|
| 0.040| 0.5384| [0.2056,0.8449]|
| 0.050| 0.5807| [0.2523,0.8587]|
| 0.060| 0.6159| [0.2949,0.8705]|
| 0.070| 0.6461| [0.3337,0.8808]|
| 0.080| 0.6723| [0.3690,0.8901]|
| 0.090| 0.6955| [0.4012,0.8985]|
| 0.100| 0.7161| [0.4306,0.9062]|
| 0.110| 0.7347| [0.4575,0.9132]|
| 0.120| 0.7515| [0.4821,0.9198]|
| 0.130| 0.7668| [0.5047,0.9258]|
| 0.140| 0.7809| [0.5255,0.9314]|
| 0.150| 0.7938| [0.5447,0.9366]|
| 0.200| 0.8454| [0.6214,0.9577]|
| 0.250| 0.8822| [0.6757,0.9723]|
| 0.300| 0.9096| [0.7160,0.9824]|
| 0.400| 0.9466| [0.7727,0.9934]|
| 0.500| 0.9691| [0.8119,0.9978]|
| 0.600| 0.9832| [0.8424,0.9994]|
| 0.700| 0.9918| [0.8684,0.9999]|
| 0.800| 0.9967| [0.8927,1.000]|
| 0.900| 0.9992| [0.9189,1.000]|
| 0.950| 0.9998| [0.9357,1.000]|

---

**Fig. 10.10** AUC determination by ROC method
295

Z(3) 0.2526 −0.3270 0.7492 1.0000
0.7325
Z(4) 0.1225 −0.7182 0.1018 0.4414
0.7325 1.0000

10.6 Mean Comparison Test—ANOVA

Analyses of variance was profounded by Prof RA Fisher to know any statistically significant differences between the means of three or more independent (unrelated) groups. This tool guides us in testing of null hypothesis and also provides a valid conclusion about sample or population (Kostrzewski 2002; Maronna et al. 2019). The following assumptions of ANOVA are considered to be tested for null hypothesis:

1. All observations are independent, identical, and homogeneous in nature.
2. Equal variance between the treatment—Homoscedasticity (tested by Bartlett’s test).
3. Experimental errors are normally distributed with mean $\mu$ and $\sigma^2$ and testing of normality by using Shapiro–Wilk test.

Test hypothesis

$H_0$ = distribution of residual = normal distribution

$H_1$ = distribution of residual ≠ normal distribution

Non-significant $p$ value = Normal distribution

$$W = \frac{\sum_{i=1}^{n} (a_i x_i^2)^2}{\sum_{i=1}^{n} (x_i - \bar{x}_i)^2},$$

$a_i$ = constants generated from the means, variances, and covariance of the order statistics of a sample of size $n$ from a normal distribution (complex)

$x_i$ = ordered sample values ($x_{(1)}$ is the smallest)

Smaller values of “$W$” are the evidence of departure from normality

The following tests has been used to test the normality of the experimental research data sets:

1. Shapiro–Wilk test—data sets mainly unique values
2. D’Agostino-Pearson normality test—lot of repeated values
3. Lilliefors normality—the mean and variance are unknown
4. Spiegelhalter’s T’ normality test—powerful test for kurtosis

10.7 Methods

Step 1: Fix the null hypothesis

$H_0$: There is no significant difference between type A and type B drug Type A = Type B

$H_1$: There is a significant difference between type A and type B drug Type A ≠ Type B

Step 2: Fix the level of significance (type I error); rejection of null hypothesis when the statement is true ($\alpha = 0.05$ or 0.01)

Step 3: Test the normality by using Shapiro–Wilk test

Step 4: Arrange the data in treatment and replication wise and compile the data from the following steps:

Between-Groups Degrees of Freedom: $df = (k - 1)$ where “$k$” is the number of groups

Within-Groups Degrees of Freedom: $df = (N - k)$ where “$N$” is the total number of subjects

Total Degrees of Freedom: $df = (N - 1)$

Sum of Squares Between Groups: $SS_B = \sum_{i=1}^{k} n_i (x_i - \bar{x})^2$ where “$n_i$” is the number of subjects in the $i$th group

Sum of Squares Within Groups: $SS_W = \sum_{i=1}^{k} (n_i - 1) S_i^2$, where $S_i$ is the standard deviation of the $i$th group

Total Sum of Squares: $SS_T = SS_B + SS_W$

Mean Square between Groups: $MS_B = SS_B / (k - 1)$

Mean Square within Groups: $MS_W = SS_W / (N - k)$

$F$-Statistic (or $F$-ratio): $F = (MS_B/MS_W)$
Step 5: If the Fisher calculated value > \( F \) table value; reject the \( H_0 \)

Illustration: The researcher wishes to test the elimination rate constant of novel regimen (pre-trial) subject to 81 cases. Experimentation was conducted in accordance with FDA guidelines. The following data sets were collected during the study intervention:

Comparison of kel–ANOVA

| Cases | Age groups |
|-------|------------|
|       | \( G_1 \) | \( G_2 \) | \( G_3 \) | \( G_4 \) | \( G_5 \) | \( G_6 \) |
| 1     | 2.1       | 3.6       | 4.1       | 3.4       | 3.77      | 3.4       |
| 2     | 2.2       | 3.5       | 4.2       | 3.3       | 3.41      | 4.1       |
| 3     | 2.3       | 3.4       | 4.3       | 3.4       | 3.78      | 4.2       |
| 4     | 2.4       | 3.7       | 4.5       | 3.7       | 3.96      | 4.7       |
| 5     | 2.5       | 3.9       | 4.3       | 3.6       | 3.58      | 3.6       |
| 6     | 2.6       | 3.6       | 4.6       | 3.5       | 3.47      | 3.5       |
| 7     | 2.8       | 3.5       | 3.8       | 3.8       | 3.62      | 3.4       |
| 8     | 2.9       | 3.8       | 3.4       | 3.4       | 3.14      | 3.8       |

ANOVA summary

| Source            | Degrees of freedom df | Sum of squares SS | Mean square MS | \( F \)-stat | \( P \) value |
|-------------------|-----------------------|-------------------|----------------|-------------|--------------|
| Between groups    | 5                     | 20.8837           | 4.1767         | 48.2568**   | 0.001        |
| Within groups     | 75                    | 6.4914            | 0.0866         |             |              |

\(^{(\text{Table } F=2.35)}\)

The results revealed that the mean elimination rate constant of group 1 (2.48 ± 0.0622); group 2 (3.48 ± 0.065); Group 3 (4.02 ± 0.099); Group 4 (3.50 ± 0.051); Group 5 (3.54 ± 0.067), and Group 6 was found to be (3.93 ± 0.117) (Fig. 10.11). The experiment was found to be statistically significant at 1% level of significance \((p < 0.01)\) (Calculated \( F > \text{table } F \) value) at error \( df \). The highest mean differences were found in Group 3 (4.02) followed by Group 6. When we wish to test any significant mean difference between the groups, we should apply the following post hoc test for mean comparison.
ANOVA: Linear Fixed Effect Model

The model is some sort of mathematical simulation, outcome results are converted to mathematical eqn to solve the clinical, biological problems ascertained with full research information. Consider panel of ANOVA linear fixed effect model from the above resulted part, the model expressed in the form of eqn is as follows:

\[ Y_{ij} = \mu + \alpha_i + \beta_j \gamma_{ij} + \epsilon_{ij} \sim N(\mu, \sigma^2) \]  

(10.19)

where
- \( Y_{ij} \) = Expected efficacy of \( i \)th drug \( j \)th subject
- \( \alpha_i \) = effect of \( i \)th drug
- \( \beta_j \) = effect of \( j \)th subject
- \( \gamma_{ij} \) = Observed value of \( i \)th drug \( j \)th subject
- \( \epsilon_{ij} \) = error associated with \( i \)th drug \( j \)th subject
- \( \mu \) = Population mean

From the above linear fixed effect model determine elimination rate constant of novel regimen, we mathematically modeled is follows from ANOVA table

\[ Y_{ij} = 3.49 + 4.17 X_{ij} + 0.086. \]

One of the variables is kept constant throughout the experimentation, by using varied parameters, we formulated ANOVA fixed effect linear model (the fixed dosage of drug employed to know the efficacy of drugs in different age groups, in this illustration we selected fixed dosage of regimen administered with different age groups of the respondents).

Linear mixed effect model is formulated based on the ANOVA salient assumptions, viz. all observations are independent and identical in nature, errors associated and normally distributed with population mean and common variance \( \sigma^2 \sim N(\mu, \sigma^2) \) (normalcy).

In case of linear fixed effect model, the factors were assigned randomly and each factor allocated mixed form to know the response variable, for example, many clinical trial linear mixed effect models are very common to know the
response parameter (McCullagh 2002; Micceri 1989; Neyman et al. 1959).

\[ Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ij} \sim N(\mu, \sigma^2), \]  
(10.20)

where \( Y_{ij} \) = Expected efficacy of \( i \)th drug \( j \)th subject

\[ Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ij} \]

\( \alpha_i \) = fixed main effect with \( \sum_{i=1}^{n} \alpha_i = 0 \)
\( \beta_j \) = random effect of \( j \)th subject
\( \alpha\beta_{ij} \) = random interaction effect
\( X_{ij} \) = Observed value of \( i \)th drug \( j \)th subject
\( \epsilon_{ij} \) = error associated with \( i \)th drug \( j \)th subject
\( \mu \) = Population mean

ANOVA table output for mixed effect model

| Source     | df | SS     | MSS     | \( F \) | \( P \) value |
|------------|----|--------|---------|---------|--------------|
| Gender     | 1  | 1594.39| 1594.39 | 17.64   | <0.001       |
| Dietary    | 2  | 1137.86| 568.97  | 8.29    | <0.001       |
| Interaction| 2  | 245.45 | 122.74  | 1.35    | >0.001       |
| Model      | 5  | 2794.86| 558.97  | 6.18    | <0.001       |
| Error      | 194| 17,529.5|90.35    |         |              |
| Total      | 199| 20,324.36|        |         |              |

Gender is fixed L: Only female population; Dietary: Various levels consider

### Numerical model

\[ Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ij} \]

\[ Y_{ij} = 102.13 + 1594.39 \times 1 + 568.97 \times 2 + 122.74 \times 1 \times 2 + 90.35 \]

\( X_1 \): At 3 months, \( x_2 = \) At 1 year cohort dietary levels (energy intake)

\[ Y_{ij} = \mu + \alpha_i + \epsilon_{ij}. \]  
(10.21)

\( \alpha_i \) = drug response of 50AKI cases in 1 month period

\( Y_{ij} \) = Expected drug response of \( i \)th drug \( j \)th AKI subject

\( \epsilon_{ij} \) = error associated with \( i \)th drug \( j \)th subject

\[ \alpha_i = (i = 1, 2, \ldots n), \alpha_i \text{ iid } \sim N\left(0, \sigma^2 \right) \]

10.10 Linear Random Effect Model

Experimenter examines the effect of some factors that are sampled from the population, e.g., there is a significant difference in drug response among 50 AKI (<50 years age) cases in 1 month duration, 50 cases were randomly selected based on the age interval and the drug response was recorded in 2-month duration. Here \( (X_1, X_2, X_3, \ldots X_n) \) random variable (patients were randomly) selected from the “\( N \)” population at different levels (levels are duration of AKI; <1 year, 1–2 years, and 3–4 years and >5 years, respectively). Mathematically linear random effect model is modeled in the following form:
Draw new “treatment effects” and new random errors.

10.11 ANOVA Repeated Measures

The repeated measures ANOVA compares means across one or more variables that are based on repeated observations. Repeated measures ANOVA can also include zero or more independent variables. Again, a repeated measures ANOVA has at least one dependent variable that has more than one observations, e.g., the researcher was interested in how two drug treatments (A&B) would also affect the level of the enzymes.

Assumptions of repeated measures

1. Observation within each treatment condition must be independent.
2. Population distribution in each treatment must be normal.
3. Variance of population distribution on each treatment must be equivalent (homogeneity).
4. Homogeneity of covariance.

ANOVA table summary

| Source    | df   | SS    | MSS   | F       | Effect size (eta square) |
|-----------|------|-------|-------|---------|-------------------------|
| Condition | SSB  | (k - 1) | 76.16 | 2.06 (p = 0.053) | 152.33/926.96 – 0.16       |
| Residual  | SSW  | (n - 1)(k) | 38.86 |         | SS by condition/SST       |
| Total     | SST  | (nk - 1) |       |         |                         |

\[
ssb = \sum \frac{T^2}{N} - \frac{G^2}{N}
\]

\[
ssT = \sum X^2 - \frac{G^2}{N}
\]

10.12 Post Hoc Test for Mean Comparison

The post hoc test is derived from the Latin word (post hoc means after this); it is to analyze the results of your experimental results. They are often tested based on family-wise errors; the probability of at least one type I error in a set of treatment comparison. The most common method of post hoc tests used for testing the pairwise treatment or subjects mean comparison is as follows:

(a) Bonferroni test
(b) Duncan multiple range test
(c) Fisher’s least significant difference (LSD)
(d) Holm–Bonferroni procedure
(e) Newman–Keuls test
(f) Rodger’s method
(g) Scheffe’s method
(h) Tukey’s test (SRD)
(i) Dunnett’s correction
(j) Benjamin–Hochberg (BH) procedure
10.12.1 Bonferroni Test

This multiple comparison post hoc correction is used in performing many independent or dependent statistical tests at the same time. The problem with running many simultaneous tests is that the probability of a significant result increases with each test run. The post hoc test sets the significance cutoff at an $\frac{\alpha}{n}$. The demerits of Bonferroni test suffer from a loss of power. The $P$ (significant event) = 1 − $P$ (no significant event) = 1 − (1−0.05)$^{25}$ = 0.92. That is almost certain (92%) that we will get at least one significant result. Example, on practical approach, the researcher testing 25 different hypotheses at the same time, using a critical value of 0.05 which is the Bonferroni correction. The simple answer $BC = \frac{\alpha}{n} = 0.05 / 25 = 0.02$. For this set of 25 tests, we would reject the null hypothesis with a probability value $p = 0.02$. Practically we cited best example of COVID19 vaccination trails intended to control pandemic of disease, at global level ongoing COVID 19 clinical trials that were reported positive interim results, the results presented in the following matrices. The researcher intended to test the Bonferroni corrections and $p$ values (Table 10.2).

| Trial phase | Primary drug(s) | Trial end date       | No of subjects (n) | Bonferroni corrections $\left(\frac{\alpha}{n}, \alpha = 0.05\right)$ | Inference                                      |
|-------------|----------------|----------------------|--------------------|------------------------------------------------------------------|------------------------------------------------|
| IV          | Adalimumab; tocilizumab | April 30, 2020    | 60                 | $P = 0.001$                                                     | 60 tests, we would reject the null hypothesis with $P = 0.001$ |
| III         | DAS-181         | April 25, 2020      | 04                 | $P = 0.013$                                                     | 4 tests, we would reject the null hypothesis with $P = 0.013$ |
| III         | Remdesivir      | April 01, 2023      | 800                | $P = 0.000$                                                     | 800 tests, we would reject the null hypothesis with $P = 0.00$ |
| III         | Remdesivir      | May 01, 2020        | 6000               | $P = 0.000$                                                     | 6000 tests, we would reject the null hypothesis with $P = 0.000$ |
| II/III      | Sarilumab       | March 09, 2021      | 400                | $P = 0.000$                                                     | 400 tests, we would reject the null hypothesis with $P = 0.000$ |
| II          | Leronlimab      | October 04, 2020    | 75                 | $P = 0.001$                                                     | 75 tests, we would reject the null hypothesis with $P = 0.001$ |
| II          | Multistem       | October 31, 2020    | 35                 | $P = 0.001$                                                     | 35 tests, we would reject the null hypothesis with $P = 0.001$ |
| II          | Bevacizumab     | June 30, 2020       | 140                | $P = 0.000$                                                     | 140 tests, we would reject the null hypothesis with $P = 0.000$ |
| II          | Nitric oxide    | December 31, 2020   | 10                 | $P = 0.005$                                                     | 10 tests, we would reject the null hypothesis with $P = 0.005$ |
| I/II        | Stem cell therapy for COVID19 | March 31, 2020 | 120                | $P = 0.000$                                                     | 120 tests, we would reject the null hypothesis with $P = 0.000$ |
**10.12.2 Duncan Multiple Range Test**

DMRT test is used to test the pairwise treatment which means comparison with control groups.

Step 1: Rank the treatment from the highest to the lowest means ($T_1$-39.30, $T_2$-20.7, $T_3$-11.3).

The next steps are to compare the H-L ($39.3-11.3 = 28$).

Step 2: Refer the $p$ value (appendix table) with treatment and error $df$.

Step 3: Find $\sigma d^2$; $\sigma d^2 = 2x$ residual mean square/\(n\).

Step 4: Take the square root of step 3.

Step 5: Multiply $\sigma d$ (step 4) by the $q$ value.

The difference between the highest and lowest means is greater than 10.50, so the highest mean is significantly different from that of the lowest mean, subsequently you should compare the second and third highest mean so on.

Step 6: Refer the $p$ value with treatment and error $df$ again follow steps 3 and 4.

The difference between the highest and lowest mean value is less than the DMRT critical value (CD) which does not differ significantly from that of lowest mean. Once you get the insignificant results, you can stop at that point.

\[
\sigma = \sqrt{\frac{2s^2}{r}},
\]

\[
R = \frac{r \cdot \sigma}{\sqrt{2}} \text{ for } p = 2,3 \ldots t.
\]
Target alpha level = overall alpha level \((0.05, 0.01)\),

\( n \) = the number of tests.

**10.12.3.1 Illustrative Example**

Use the HB method to test the following four hypotheses and their associated \( p \) values at an alpha level of 0.05

\[
\begin{align*}
H_1 &= 0.01 \\
H_2 &= 0.04 \\
H_3 &= 0.03 \\
H_4 &= 0.005
\end{align*}
\]

Step 1: Order the \( P \) values from the smallest to the greatest.

\[
\begin{align*}
H_4 &= 0.005 \\
H_1 &= 0.01 \\
H_3 &= 0.03 \\
H_2 &= 0.04
\end{align*}
\]

Step 2: Calculate the HB formula for the first rank.

\[
\text{HB} = \frac{0.05}{4 - 2 + 1} = \frac{0.05}{3} = 0.0167.
\]

Step 3: compare the first ranked (smallest) \( p \) value from step 1 to the alpha level calculated in step 2.

The smallest \( p \) value, in step 1 \((H4:0.005)<\alpha\) level in step 2 \((0.0125)\). If the \( p \) value is smaller, reject the null hypothesis for this individual test. The \( P \) value of 0.005 is less than 0.0125 so that the null hypothesis for \( H_1 \) is rejected.

Step 4: Repeat the HB formula for second rank.

\[
\text{HB} = \frac{0.05}{4 - 2 + 1} = \frac{0.05}{3} = 0.016.
\]

Compare the results from the formula in step 4 to the second ranked \( p \) value \((H_1 = 0.01 < 0.0167)\) so that \( H_1 \) is rejected.

**10.12.4 Least Significant Difference Test (LSD)**

The LSD test is the simplest and the most commonly used post hoc test in agricultural, animal, and medical researches. LSD procedure for making the pair comparisons. The procedure provides a single LSD value, at a prescribed level of significance, which serves as the boundary between significant and insignificant differences between any pair of treatment means. The test is most appropriate for making planned pair comparison but not valid for comparing all possible pairs of means, especially when the number of treatment group is large.

Step 1: Compute the mean difference between the \( i \)th and \( j \)th treatment as follows:

\[
d_{ij} = \bar{x}_i - \bar{x}_j.
\]

Step 2: Determine the LSD value at the desired level of significance.

\[
\text{LSD} = t_{\alpha = 0.05, 0.01} \times S_d, \]

where \( S_d \) is the standard error of mean difference, \( t_{\alpha = 0.05, 0.01} \) is the table value from appendix

\[
S_d = \sqrt{\frac{2 \text{EMS}}{r}},
\]

where “\( r \)” is the number of replications.
### 10.12 Post Hoc Test for Mean Comparison

|                  | df | SS   | MSS  | F         | P value |
|------------------|----|------|------|-----------|---------|
| Between group    | 3  | 419.20 | 181.21 | 6.45 (Table F=2.63) | 0.0013  |
| Within the group | 36 | 959.58 | 26.65 |           |         |

\[
\text{LSD} = 2.08 \times \sqrt{\frac{2 \times 26.65}{10}} = 4.68
\]

\[
d_j = |\bar{X}_j - \bar{X}| \geq \text{LSD} \text{ reject } H_0
\]

#### 10.12.5 Newman–Keuls Test

The Newman–Keuls test is similar to post hoc test for Tukey’s to identify sample means that are different from each other. Newman–Keuls uses different critical values for comparing different pairs of means. Therefore, it is more likely to find significant differences, e.g., we fix the \( H_0 \): mean efficacy of Type A drug = mean efficacy of Type B drug where type A and B could be any possible pair.

Step 1: Order means from largest to smallest, compare differences between the groups with the largest mean to the group with the smallest mean.

Step 2: Determine the SE using the MSE (from ANOVA input).

\[
S_{AB} = \sqrt{\frac{\text{MSE}}{n}} \quad \text{equal sample size; if the sample sizes are not equal} \quad S_{AB} = \sqrt{\frac{\text{MSE}}{2} \left( \frac{1}{n_a} + \frac{1}{n_b} \right)}.
\]

Step 3: Compute \( q \) value

\[
q = \left[ \frac{(\bar{X}_A - \bar{X}_B)}{S_{AB}} \right].
\]

Refer the \( q \) critical value from a table. The rows are the number of means being compared (i.e., number of treatment) and the columns are the \( df \). If the calculated “\( q \)” value is more than the table value, then rejects the \( H \) if the two means are equal, stop the test, you can conclude that there is no significant difference between any pairs of means. If the two means are unequal, repeat the steps.

#### 10.12.6 Scheffe Test

It is coined by American Statistician Henry Scheffe, post hoc test used in analysis of variance. After we run the ANOVA, we got a significant \( F \) statistics (reject the \( H_0 \)). We should apply the Schiff’s test to know the pairwise means significant. However, the Scheffe’s test corrects alpha for simple and complex means comparisons. Complex means comparison involve comparing more than one pair of means simultaneously, most flexible and also the test with lowest statistical power “\( \beta \)”. In a practical point of view, if the researcher wants to make pairwise comparison, run the Tukey’s test because it will have narrowed CI-95% or 99% or else if you want to compare all possible simple and complex pairs of means, run the Scheffe test as it will have a narrowed CI. Only run this test if you have rejected \( H_0 \) in an ANOVA test, indicating that the means are not the same. Otherwise, the means are equal and so there is no point in running.

The null hypotheses for the test are that all means are the same \( H_0: \mu = \mu_0; H_0 : \text{The mean efficacy is no difference between drug A and drug B} \)
Step 1: Calculate absolute values of pairwise comparisons \( \overline{X}_{AB} = 36.00, \overline{X}_{AC} = 34.50, \overline{X}_{AD} = 36.60, \overline{X}_{BD} = 36.21 \); \( F \) value from ANOVA (3.6 \( df \) at 0.05), MSE 0.028.

| Drug | AB | AC | AD | BC | BD | CD |
|------|----|----|----|----|----|----|
| Mean difference | 1.50 | 0.40 | 0.21 | 1.1 | 1.71 | 0.61 |

Step 2: Apply the following formula to find a set of Scheffe’s formula values.

\[
S_c = \sqrt{(k-1) F \text{ value} \cdot \text{MSE} \left( \frac{1}{n_i} + \frac{1}{n_j} \right)},
\]

where \((k - 1)\) is the between sample \(df\) \(F\), \(1 \leq n - k; k\); \(\alpha\) is the \(f\) value (from ANOVA(3.24))

\[
\text{MSE} = \text{mean square error}, \text{e.g., comparing A and B drugs}
\]

\[
\sqrt{3 \times 3.24 \times 0.08 \times \left( \frac{1}{5} + \frac{1}{4} \right)} = 0.32.
\]

Follow steps 1 and 2. Find any values that are larger than the comparison values. Larger values are statistically significant.

### 10.12.7 Turkeys’ or Honest Significant Difference Test

The Tukey’s posttest is only applicable to smaller treatment groups (3–6), when “\(F\)” is significant we want to compare the mean differences between and within the groups. The post hoc test determined based on the studentized range distribution. The test compares all possible pairs of means, we should test all possible pairs among means using the Tukey’s HSD, calculate HSD for each pair of means using the following formula:

\[
\text{HSD} = \frac{M_i - M_j}{\sqrt{\text{MS}_w / n_h}},
\]

where \((M_i - M_j)\) is the difference between the pairs of means; \(\text{MS}_w\) = mean square within

Step 1: Perform the ANOVA test.

Step 2: Choose two means from the ANOVA output (means, MSE, number of treatment, \(df\) within).

Step 3: Calculate the HSD statistics for Tukey’s test using the formula.

Step 4: Find the score in Turkeys’ critical value table.

Step 5: Compare the score. If the calculated value >\(CD\), the two means are statistically significant.

### 10.12.8 Dunnett’s Test

Dunnett’s test is the most appropriate test to compare means from several experimental groups against control group mean to know there is a difference. We can consider fixed control group varying with experimental group means.

\[
D_{\text{Dunnett}} = t_{\text{Dunnett}} \sqrt{\frac{2\text{EMS}}{n}}; \text{ EMS = error mean square, } n = \text{number of sample}.
\]

Step 1: Refer the \(t_{\text{Dunnett}}\) critical value in the Dunnett critical value table (0.05;0.01 \(n = \text{sample size 5, 20 df}\)).
\[ D_{\text{Dunnett}} = 2.65 \sqrt{\frac{2 \times (15.39)}{5}} = 6.57. \]

Critical \( D_{\text{Dunnett}} \) value is 6.57; If the distance between a control group mean and an experimental group mean is greater than 6.575, then that distance is significant.

### 10.12.9 Benjamini–Hochberg Test

The BH test is a powerful method that decreases the false discovery rate. Adjusting the rate helps to control for the fact that sometimes small \( p \) values (<5%) happen by chance, which could lead you to incorrectly reject the \( H_0 \). In other words, the BH test helps us lead to avoid type error (false positive test). A \( P \) value of 5% means that there is only a 5% chance that we would get observed result if the \( H_0 \) is true. Since it is only probability many times, true hypothesis is thrown out because of the randomness of results. The concrete example we had is a group of 100 patients who are free from certain disease. Our Ho is that patients are free of disease and our alternate is that they do have the disease. We apply 100 statistical tests at the 5% level, roughly 5% of our report as false positives. In this paradigm, BH test will decrease the number of false positives.

#### Steps

1. **Step 1:** Order the individual \( p \) values in ascending order.
2. **Step 2:** Assign ranks to the \( P \) value (e.g., smallest has a rank of 1, the second smallest has a rank 2).
3. **Step 3:** Calculate each individual \( p \) values Benjamini–Hochberg critical value using the formula.

\[
CD = (i/m)Q; \quad i = \text{the individual } p \text{ value’s rank}, \quad m = \text{total number of tests}, \quad Q = \text{the false discovery rate (a percentage, chosen by you)}. 
\]

Compare the original values to the critical BH from the above step; find the largest \( p \) value, i.e., smaller than the CD value.

| Variable               | \( p \) value | Rank | \( CD = (i/m)Q \) |
|------------------------|---------------|------|-------------------|
| Depression             | 0.001         | 1    | 0.01              |
| Family H/o             | 0.008         | 2    | 0.02              |
| Obesity                | 0.039         | 3    | 0.03              |
| Other health problem   | 0.041         | 4    | 0.04              |
| Children               | 0.042         | 5    | 0.05              |
| Divorce                | 0.060         | 6    | 0.06              |
| Death of spouse        | 0.074         | 7    | 0.07              |
| Limited income         | 0.205         | 8    | 0.08              |
| **CD**                 | **0.042**     |      |                    |

The bolded \( p \) value is the highest \( p \) value, i.e., also smaller than the CD: 0.042 < 0.050 consider as significant.
### 10.13 Least Square Estimation Method of AUC

The least square estimation of AUC is an easiest method and more robust in nature, select the data points with respect to plasma concentration and time in hours. The data \((X,Y)\) distributed in a ring shape on \(XY\) plane, the least square regression employed to determine the equation of a circle that will best fit with available data points, the regression helps to calculate parameters of \(k, m,\) and \(r\) values of the curve (Lemke et al. 2008; Laudisio et al. 2009).

\[
(x-k)^2 + (y-m)^2 = r^2. \tag{10.22}
\]

Minimizing the above eqn, the eqn becomes

\[
F((k,m,r)) = \sum ((xi-k)^2 + (yi-m)^2 - r^2)^2. \tag{10.23}
\]

The eqn of the circle is liberalized by the model \(F/\partial k = 0, \partial F/\partial m = 0,\) and \(\partial F/\partial r = 0\) we obtain the following eqn:

\[
(x-k)^2 + (y-m)^2 = r^2,
\]

\[
(x^2 - ker + k^2 + y^2 - 2my + m^2 = r^2),
\]

\[
X^2 + y^2 = 2ker + 2my + r^2 - k^2 - m^2.
\]

These results tend to linear eqn with the coefficients of \(A, B,\) and undetermined or no estimation. As such we can use the matrices to solve the least square problem. Once we obtain \(A, B,\) and \(C\) with matrices. The circle regression model is supported by the following matrix eqn:

\[
\begin{bmatrix}
\sum x_i^2 & \sum x_iy_i & \sum x_i \\
\sum x_iy_i & \sum y_i^2 & \sum y_i \\
\sum x_i & \sum y_i & n
\end{bmatrix}
\begin{bmatrix}
A \\
B \\
C
\end{bmatrix}
= \begin{bmatrix}
\sum x_i(x_i^2 + y_i^2) \\
\sum y_i(x_i^2 + y_i^2) \\
\sum x_i^2 + y_i^2
\end{bmatrix},
\]

where “\(n\)” is the number of data points \((X_i,Y_i)\) of time and plasma concentration. In such situation we use \(3 \times 3\) matrix outlined in the left is invertible. The \(A, B,\) and \(C\) are unique and this was determined as the best circle, subsequently determined the \(k, m,\) and \(r\) parameters (Fig. 10.12).

\[
k = \frac{A}{2}, m = \frac{B}{2} \text{ and } r = \left(\sqrt{4c + A^2 + B^2}\right)/2.
\]

Find out the slope and intercept from the nearest point of circle and draw the tangent from the nearest data points and intercept, and mark the CP0,CP1. Cp last point from the trapezoid rule

\[
\text{Total AUC}_{0-\infty} = \left\{ \frac{CP_0 + CP_1}{2} t_1 + \frac{CP_1 + CP_2}{2} t_2 - t_1 \right\} + \frac{CP_2 + CP_3}{2} t_3 - t_2 \ldots \frac{CP_{\text{last}}}{\text{kel}}.
\]

#### Least-squares circle calculator—results

| Sample size: | 6 |
| Center \((x,y):\) | \((15.0550, 8.8361)\) |
| Radius: | 20.8507 |
| Best Fit Circle Equation: | \((x - 15.0550)^2 + (y - 8.8361)^2 = 20.8507^2\) |

### 10.14 Quadratic Regression Modeling

More advanced tool for extrapolation of AUC under quadratic modeling techniques is more applicable. The model identifies the parabola set of data in clinical trial. The kel is derived biologically and differs from many confounders; it
is an extension of linear regression, the results of the model will predict about various data points (Royston and Sauerbrei 2008; Shmueli 2010). However, the measurement errors were taken from both the variables. It is used to analyze how differences in one variable can be explained by a difference in a second variable. For example, when a woman gets pregnant has a direct relation to when they give birth, so $R$ square would be close to 100%. On the other hand, $R$ square would be practically zero for when woman gets pregnant and when she throws a retirement party for a parent.

Quadratic regression: $y = ax^2 + bx + c$, where $a \neq 0$

Coefficients $(a, b, c)$

$$a = \frac{\left(\sum x_i^2\right)^2 \sum x_i y_i - \sum x_i \sum x_i^2 y_i \sum x_i^3 + \sum x_i \sum x_i^4 \sum y_i + \left(\sum x_i^2\right)^2 \sum x_i y_i - \sum x_i^2 \sum x_i^3 + \sum x_i \sum x_i^4 \sum y_i}{\left(\sum x_i^2\right)^3 - 2 \sum x_i \sum x_i^2 \sum x_i^3 + n \left(\sum x_i^2\right)^2 + n \left(\sum x_i\right)^2 \sum x_i^4 - n \left(\sum x_i^2 \sum x_i^4\right)}$$

$$b = \frac{n \sum x_i y_i \sum x_i^2 y_i - \sum x_i \sum x_i^2 \sum x_i^3 y_i + \left(\sum x_i^2\right)^2 \sum x_i y_i \sum x_i \sum x_i^4 + \sum y_i - n \sum x_i^4 \sum x_i y_i - \sum x_i^2 \sum x_i^3 \sum y_i}{\left(\sum x_i^2\right)^3 - 2 \sum x_i \sum x_i^2 \sum x_i^3 + n \left(\sum x_i^2\right)^2 + n \left(\sum x_i\right)^2 \sum x_i^4 - n \sum x_i^2 \sum x_i^4}$$
\[
c = \frac{\left(\sum x_i^2\right)^2 \sum x_i^2 y_i - n \sum x_i^2 y_i - \sum x_i \sum x_i^2 + \sum y_i + n \sum x_i^2 \sum y_i + \left(\sum x_i^2\right)^2 \sum y_i - \sum x_i \sum x_i^3 \sum y_i}{\left(\sum x_i^2\right)^3 - 2 \sum x_i \sum x_i^2 \sum x_i^3 + n \left(\sum x_i^2\right)^2 + n \left(\sum x_i^2\right)^3 - n \sum x_i^2 \sum x_i^4}
\]

Mean: \(x = \frac{\sum x_i}{n};\) Mean: \(y = \frac{\sum y_i}{n}\)

Correlation coefficient “r” is given by the following:

\[
r = \frac{\sum (y_i - (ax_i^2 + bx_i + b))^2}{\sum (y_i - \bar{y})^2}
\]

where “n” is the total number of samples, \(x_i (x_1, x_2, ..., x_n)\) are the x values and \(y_i (y_1, y_2, ..., y_n)\) are the y values; \(\sum x_i\) is the sum of x values; \(\sum y_i\) is the sum of y values; \(\sum x_i y_i\) is the sum of products of x and y values; \(\sum x_i^2\) is the sum of squares of x values; \(\sum x_i^3\) is the sum of the cubes of x values; \(\sum x_i^4\) is the sum of the fourth powers of x value.

Illustration: The following data sets were collected from drug trial experiment with respect to hours and plasma concentration. We should apply the Quadratic regression model for AUC calculation.

| X: (hours) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | −1  | −2  | −3  | −4  |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Y: (Plasma concentration) | 80  | 81  | 88  | 74  | 68  | 55  | 40  | 25  | 20  | 15  | 10  | 9   | 15.19 | 10.14 | 24.04 | 13.05 | 15.19 |

10.14.1 Model Results

Regression data

| x   | y    | xy   | x²   | x³   | x⁴   | x²y   |
|-----|------|------|------|------|------|-------|
| −5  | 15.89| −79.45| 25.00| −125.00| 625.00| 397.25|
| −4  | 15.19| −60.76| 16.00| −64.00| 256.00| 243.04|
| −3  | 13.05| −39.15| 9.00 | −27.00| 81.00 | 117.45|
| −2  | 9.81 | −19.62| 4.00 | −8.00 | 16.00 | 39.24 |
| −1  | 12.01| −12.01| 1.00 | −1.00 | 1.00  | 12.01 |
| 0   | 9.35 | 0.00  | 0.00 | 0.00  | 0.00  | 0.00  |
| 1   | 8.62 | 8.62  | 1.00 | 1.00  | 1.00  | 8.62  |
| 2   | 13.11| 26.22 | 4.00 | 8.00  | 16.00 | 52.44 |
| 3   | 12.16| 36.48 | 9.00 | 27.00 | 81.00 | 109.44|
| 4   | 15.57| 62.28 | 16.00| 64.00 | 256.00| 249.12|
| \(\sum x_i = −5.00\) | \(\sum y_i = 124.76\) | \(\sum x_i y_i = −77.39\) | \(\sum x_i^2 = 85.00\) | \(\sum x_i^3 = −125.00\) | \(\sum x_i^4 = 1333.00\) | \(\sum x_i^2 y_i = 1228.61\) |
Quadratic regression—results

| Sample size: | 10 |
|-------------|----|
| Mean $x$:   | $-0.5000$ |
| Mean $y$:   | $12.4760$ |
| $a$:        | $0.2900$ |
| $b$:        | $0.1081$ |
| $c$:        | $0.0647$ |

Regression equation: $y = 0.2900x^2 + 0.1081x + 10.0647$

Correlation coefficient ($r$): $0.8763$

Total $\text{AUC}_{0-\infty} = \left\{ \frac{\text{CP}_0 + \text{CP}_1}{2} t_1 + \frac{\text{CP}_1 + \text{CP}_2}{2} t_2 - t_1 \right\} + \frac{\text{CP}_2 + \text{CP}_3 - t_2 \ldots \text{CP}_{\text{last}}}{\text{kel}}.$ \hfill (10.24)

10.15 Rational Function Regression

The ratio of two linear functions represents one of the most straightforward rational functions. Rational function of ‘$Y$’ can take in the form of $Y = (ax + b)/(x - b)$ represent a good method of modeling any data that levels offer a given time period without any oscillations. The horizontal system asymptote of a rational function is $y = a,$ while the vertical asymptote is $x = b$ and $y$ intercept is $c/bowmen$ function takes the form $y = (ax + c)/(x - b),$ the $a,$ $b,$ and $c$ parameters are not linear. Since it is possible to transform the eqn through the use of simple mathematical form

\[
y = \frac{(ax + c)}{x - b},
\]

\[
(x - b)y = ax + c,
\]

\[
xy - by = ax + c,
\]

Fig. 10.13 AUC by quadratic regression method
\[ xy = ax + by + c. \]

This above eqn does not incorporate linear \( a, b, c \) variables. As such that we apply the least square method to identify the “best fit” values \( a, b, \) and \( c \), i.e., we minimize the eqn as follows:

\[
F(a,b,c) = \sum_{i=1}^{n} \left( x_i y_i - ax_i - bx_i - c \right)^2. \tag{10.25}
\]

This is tantamount to solving the system as follows:

\[
\frac{\partial F}{\partial a} = 0, \frac{\partial F}{\partial b} = 0 \text{ and } \frac{\partial F}{\partial c} = 0.
\]

The solution can be determined using matrix math. Using matrices to determine \( a, b, \) and \( c \).

The matrix equation that can be employed for simple rational regression is as follows:

\[
\begin{bmatrix}
\sum x_i^2 & \sum x_i y_i & \sum x_i \\
\sum x_i y_i & \sum y_i^2 & \sum y_i \\
\sum x_i & \sum y_i & n
\end{bmatrix}
\begin{bmatrix}
a \\
b \\
c
\end{bmatrix} =
\begin{bmatrix}
\sum x_i^2 y_i \\
\sum y_i^2 \\
\sum x_i y_i
\end{bmatrix}
\]

where “\( n \)” is the number of data pairs \((x_i, y_i)\).

Providing the three-by-three matrix presented on the left is invertible, a unique solution \((a, b, c)\)

will be employed to minimize the function \( F(a, b, c) \) and delivers the parameters for the best fit rational function.

**Example**

Identify the equation of a rational function that fits the points \((x, y)\):

\[
(4.3), (2.4), (3.6)
\]

Through the use of this rational function regression calculator, you can delineate the following equation:

\[
y = \left(3.6x - 12\right)/(x - 3.2).
\]

This equation represents a reasonably good fit for the data.

Robustness of the model tested by regression function:

| \( X \) (hours) | \( Y \) (plasma concentration) |
|-----------------|---------------------------------|
| 1               | 4.11                            |
| 2               | 4.56                            |
| 3               | 5.59                            |
| 4               | 7.97                            |
| 5               | 8.25                            |

| Sample size: 5 |
|----------------|
| \( a \): 4.6731 |
| \( b \): 4.3036 |
| \( c \): -19.628 |

**Rational function regression**

\[
y = (4.6731x - 19.628)/(x - 4.3036)
\]

**10.16 Brownian Drug Diffusion Stochastic Model**

The movement of drug particles from the site of application to the circulation and the rate and extent at which the drug particles go to the systemic circulation is known as blood absorption. However, the rate and efficiency absorption depend on the route
of administration. A route of administration in pharmacology and toxicology is the path by which a drug taken into the body. Various routes of administration, oral route sublingual or buckle route, rectal route, parental, nasal inhalation, and topical route. The drug diffusion takes place in the body at different mechanisms like passive diffusion, i.e. driving force concentration gradient high to low (Brownian motion principle) until equilibrium exists. Once absorption process will be completed, the drug has been utilized and diffused in different body tissues. The passive diffusion does not involve a carrier, and the process is not saturable (Chandrasekhar 1943). The rate of absorption depends on lipophilicity (lipid–water partition coefficient) which determines how fast the drug gets absorbed. Lipid-soluble drugs movement between compartments is dependent on the concentration difference between them, the process is exponential and the rate of transfer to the slower tissues decreases as they accumulate more drug and readily move across biological membranes, whereas water-soluble drugs penetrate the cell membrane through aqueous channel depending on their chemical properties, drug absorption can occur from GI tract either by passive diffusion or by active transport. However, carrier-mediated absorption is appear to present either an import or an export site to the transported molecule and undergo substrate-induced conformational change, is likely that some kinds of drugs are absorbed by this process with the help of carrier. In this cited process, a carrier molecule will make a complex with the drug. Usually polar molecules are absorbed by this process (hydrophobic coating). Many factors will affect the drug absorption concentration of drug molecules, as most of drugs are absorbed by passive diffusion concentration and is very important. High concentration will be applied for better activity, e.g., lidocaine local anesthesia (2.0%) solution will produce anesthesia more rapidly than 0.20% solution of lidocaine if the amount is same. PK model derivatives is mostly applicable when solution drug is applied, i.e., physical state of drug, some of the drugs are solid, some are liquid, suspension, emulsion, solution, and tablets or capsules. Since before absorption, the drug must be in solution so drug in the solution or liquid dosage form is absorbed faster than solid dosage form, e.g., tablet must be disintegrated, dissoyble than the whole process but for liquid or solution dosage form there is no need to disintegrate or dissolution. Viscous product absorption will be slower than the diluted solution surface area of the absorptive site; higher the surface area, greater will be the absorption and better action. Large intestine, pulmonary alveoli, skin if high concentration of drug. Biologically the rate of administration is very important to know the level absorption in different tissues basically IV route no absorption required or no loss of drug blood circulation of the absorptive site. If the blood circulation of the absorptive site is maximum or efficient, the absorption will be faster. Blood circulation fastens the absorption. In morphine toxicity with respect toAnimal and human studies indicate the existence of important sex differences in opioid-induced antinociception and analgesia. We observed greater morphine analgesia in women compared to men, which was related to sex differences in morphine pharmacodynamics and not to differences in its pharmacokinetics, because we believe that sex dependency in opioid behavior is probably not restricted to morphine but may be an inherent property of opioid analgesics, it is of interest to assess the existence of a sex-specific dichotomy in M6G analgesia and to quantify whether such an observation is related to M6G pharmacokinetics, pharmacodynamics, or both Gastric emptying is the time how quickly the drug leaves the stomach. As we glorify surface area of the stomach, it is smaller than intestine, so most drugs are absorbed in the intestine. So, absorption is inversely proportional to gastric emptying. The presence of food will dilute the drug particles and decrease the absorption level. As a result, drug will be absorbed slower compared to that when there is no food. Another factor of drug absorption is bioavailability; it is the fraction of administered drug that reaches the systemic circulation (Setiawati et al. 2009; Shargel et al. 2005; Turnheim 2003). Typically, Bioavailability expressed the fraction of administered drug that gains access to the systemic circulation in chemically unchanged form, e.g., if 100 mg of a drug is administered orally, 70 mg of this absorbed drug is unchanged, hence the bioavailability is 70%. In case of bioavailability first-pass hepatic metabolism when a drug is absorbed across the GI tract, it enters the portal circulation before entering the systemic circulation. If the drug is rapidly metabolized by the liver, the amount of unchanged drug that gains access to the systemic circulation is decreased, e.g., propranolol or lido-
caine. The solubility of the drug will depend on the type of drugs, in case of hydrophilic drugs are poorly absorbed because of their inability to cross the lipid-rich membranes. Paradoxically, drugs that are extremely hydrophobic are also poorly absorbed because they are totally insoluble in the aqueous body fluids and therefore cannot gain access to the surface of cells (Tsang and Gerson 1990; Vogels et al. 2007; Winter 2004). For a drug to be readily absorbed, it must be largely hydrophobic yet have some solubility in aqueous solutions. Chemical instability is important derivatives in PK process, it involve alterations in the molecular structure producing a new chemical entity, by bond formation or cleavage, e.g., insulin, nitroglycerine, and penicillin G. The nature of drug formulation depends on particle size, salt form, binders and dispersing agents, etc.

Drug diffusion is a degree of binding the drug to the plasma, and the tissue proteins and the relative hydrophobicity/lipophilicity of the drug. Their concentration varies from compartment to compartment. Usually it is considered to be operated by diffusion. But it is arbitrary. The drug concentration in all cell/compartments is not equal. Exception, ethanol is distributed equally. Drug distribution is the paradoxical changes in human body system. However, the drug is extensively distributed in the body, which means a relatively low concentration of plasma drug. Movement of drug proceeds until an equilibrium is established between unbound drug in plasma and tissue fluids. Protein binding describes the ability of proteins to form bonds with other substances, and most commonly refer to the bonding of drugs to molecules in drug plasma (red blood cells, other components of the blood, and to tissue membranes), that affect distribution of the drug depends with the affinity of the drug toward protein. One of the best eg., the Acidic albumin basic glycoprotein sulfamethoxazole (30%) bound and (90%) bound if protein binding is very high, volume of drug distribution will be very low because protein bound drug cannot overcome many barriers by diffusion. Sometimes protein binding acts as a reservoir and will not exert action. Extensive binding may prolong the action of drug. The next step of drug process is metabolism or biotransformation which is a chemical alteration of drug in the body. It is needed to render nonpolar compounds so that they are not reabsorbed in the renal tubules and are excreted (Fig. 10.14). Most hydrophilic drugs are not biotransformed and are excreted unchanged. Many pharmacologists attempt to know the diffusion mechanism by stochastic process, since invariably due to paucity of literature, they are unable to find the suitable model for extrapolation of time taken for diffusion from intestine, arteries, and other human body systems. In this pragmatic research gap, the present section tries to explore and demonstrate new stochastic model of drug diffusion process from Brownian motion principles.

The diffusion rate of a liquid process is much slower than gas because its bioavailability is more,

**Fig. 10.14** Process of drug absorption and diffusion
but faster than solids due to the close association of molecules and lower kinetic energy because of stronger intermolecular attractive forces between the molecules compared with gases. Diffusion is the mixing of particles, i.e., movement of particles from an area of higher concentration to an area of lower concentration. From the above-cited principles of brownian motion, we assumed that the diffusion of drug substances, either liquid or solid, can take place in human body from an area of higher concentration to the area of lower concentration and also uniformly distributed over a period of time (Korkushko et al. 1984). The inclusion of the above-said Brownian principles, the present study formulated the stochastic model to know the diffusion rate of drug in varied time interval in association with various organs of the human body (Fig. 10.15).

On pharmacological intervention, the diffusion of drug will depend on the bioavailability and plasma concentrations. It is the net movement of drug particles (molecules) from a region of high concentration to a region of low concentration (e.g., small intestine, arteries vein to various other organs) due to random movement of blood. However, such kind of diffusion process can be involved in various biological mechanisms of the movement of blood in the opposite direction. Let us consider movement of random diffusion process $X_1, X_2, \ldots X_n$ be the random variable which can assume Gaussian distribution $x_i \sim N(\mu, \sigma^2)$, every individual process of diffusion rate is i.i.d’s and homoscedasticity (equal variance). The variable of diffusion process was simulated Thompson iteration or Runge and Kutta method (statistical outcome of random Brownian motion and eventually will result in similar concentrations throughout the solution). Various diffuse fluxes of first and second kinds were obtained from the Stokes-Einstein Eq. (10.27) ($J$, the amount of substance moving through a unit area as per unit time $t$) is governed by Flick’s first law (Fig. 10.16).

The intervention of change in the diffusion of drug with respect to time “$dt$” is given by the following formula:

$$\frac{Df}{dt} = -DA \frac{d\omega}{dx},$$

$$J = -D \frac{d\omega}{dx},$$
where “d” is the diffusivity \( (m^2 \times S^{-1}) \), \( d\omega \) is the change in drug concentration, and \( dx \) is the change in time \( (m) \). The diffusion direction is from higher concentration to lower blood concentration such that \( \frac{df}{dt} \) is always negative and the diffusive flux is always positive in the diffusion direction. Thus, drug particle flux is proportional to the concentration gradient. As per the Einstein definition, the diffusion of drug is modeled by the following eqn:

\[
\text{Diffusion}(D) = \lim_{t \to \infty} \frac{[r(t) - r(0)]^2}{6ti}, \quad (10.27)
\]

where “t” is the time taken for diffusion \( (t = 0, 1, 2, 3, \ldots, \text{ith hours}) \).

In a diffusive process, the concentration of substance in the region of higher concentration gradually decreases and the concentration of the substances in the region of lower concentration gradually increases. With time, the concentration gradient dissipates within the bulk. Diffusion increases entropy (randomness) leading to a lower energy state. Eventually, equilibrium is established with uniform distribution throughout (Uniform distribution of drug substances throughout the body). The concentration change with time is described by Fick’s second law:

\[
\frac{df}{dt} = D \frac{d^2\omega}{dx^2}.
\]
“$D$” is described by the **Stokes-Einstein eqn** for translational diffusion state.

$$D = \frac{K_B T_{\text{max}}}{6\pi \eta r} = \frac{C_{\text{max}} \cdot \text{Time}}{N} \cdot \frac{1}{6\pi \eta r}. \quad (10.28)$$

- $K_B$ = Boltzmann constant
- $T_{\text{max}}$ = Maximum time taken for drug diffusion to reach the threshold level
- $N$ = Number of substances
- $C_{\text{max}}$ = Maximum plasma concentration
- $\eta$ = Viscosity of the blood
- $r$ = Average drug particle size; $\pi = 3.12$

(Fig. 10.17)

### 10.16.1 Numerical Results

**Diffusion rate initial stage fluxes** = 5.58252375

- **Blood volume** = 150–272.04
- **Total duration** ($t$) **hours** = 3.36
- **Convexity of the curve** = 15.64

| Organ                                      | Lower bound motion | Upper motion |
|--------------------------------------------|--------------------|--------------|
| Arteries vein to other tissues fluxes      | 1.64               | 2.73         |
| diffusion (hours)                          |                    |              |
| Particle size (nano)                       | 0.01–0.03          |              |
| Percent of diffusion rate at first wave    | 33–60%             |              |

100% absorption takes place in small intestine with mean flux time 25–40 minutes

**Fig. 10.17** Diffusion process estimated by Brownian motion for the first wave (1–2.5 h)

### 10.17 Brownian Random Walk Model

A random walk, sometimes also called a “drunkard walk,” the first step of the model helps us in understanding the Brownian motion and drug diffusion process on a random basis (Chandrasekhar 1943; Royston and Sauerbrei 2008). A random walk is formalization of scientific intuitive idea of taking successive steps. The simplest random walk is a path construction according to the diffusive process of drug, plasma concentration, bioavailability, and volume of the blood, respectively. We consider random variable of $X_1, X_2, \ldots, X_n$ follows with drunkard walk $W_n$. On each diffusion rate $D_1, D_2, \ldots, D_k$ is strongly associated with Brownian motion with movement from higher concentration to the region of lower concentration. Since each walk was derived based on the Gaussianity and following random process model,
\[ X_n = \sum_{i=1}^{n} W_n D_k, \quad (10.29) \]

\[ D_k \begin{bmatrix} 1 \quad p = 1/2 \\ -1 \quad p = 1/2 \end{bmatrix}, \]

\[ D_k = \pm \sqrt{\frac{T}{n}} E(D_k) = 0, \]

\[ E(D_k^2) = \frac{T}{n}. \]

\[ E(D_k D_l) = E(D_k) E(D_l). \]

The layer of each successive jumps or fluxes is equal to \( \left( \frac{T}{n} \right) \) with equal probability.

In other words, if we consider a sequence of independent binomial \( X_i \) taking values +1 or -1 with equal probability \( p = q = 0.50 \), then the value of random walk at the \( i \)th flux in Brownian motion is defined recursively as follows.

\[ W_n D_k \sim B(n, p, q), \quad (10.30) \]

where “\( n \)” be the fixed dose of drug with equal probability taking “\( p \)” as the diffusion flux higher concentration and “\( q \)” as the diffusion flux at lower concentration \( p + q = 1/2 \). Then \( p, q, n_1, n_2 \) and \( N \) are related by the following eqn:

\[ \sum_{d=1}^{J} \frac{d}{(J + d)! (J - d)!} = \frac{1}{2 \Gamma (J) \Gamma (1 + J)} \]

\[ \frac{N!}{2^N} \left[ \sum_{d=-J}^{-(J-1)} \frac{|2d|}{(2J + 2d)^J} \right] + \frac{N!}{2^N} \left[ \sum_{d=1,2,...} \frac{|2d|}{(2J - 2d)^J} \right] \]

\( (p + q) = 1 \) and \( n_1, n_2, n_k \) is the diffusion fluxes \( n_1 + n_2 + \ldots + n_k = N_k \).

We examine the probability of taking exactly \( n_1 \) diffusion flux out of “\( N \)” to the right, the model becomes \( \binom{N}{n_1} = \binom{n_1 + n_2}{n_2} \) ways of taking \( n_1 \) steps to higher concentration and \( n_2 \) to the lower concentration, where \( \binom{n}{m} \) is the binomial coefficient. The probability of taking particular ordered sequences \( n_1 \) and \( n_2 \) diffusion fluxes \( p^n q^{n_2} \). Therefore, the \( p(n_1) \) diffusion is

\[ P(n_1) = \frac{(n_1 + n_2)!}{n_1! n_2!} \quad p^{n_1} q^{n_2} = \frac{N!}{n_1! (N - n_1)!} p^{n_1} q^{N - n_1}. \]

10.31

where \( n! \) is a factorial. However, this is simply a BD, so the mean number of fluxes \( n_1 \) is the higher concentration plasma flow and the mean number of steps to the lower level is \( n_1 = N - n_1 = N (1 - p) = qN \).

Similarly the variance of each diffusion was calculated by

\[ \sigma_{n_1}^2 = (n_1^2) - (n_2^2) = N p q, \]

\[ \sigma_{n_1} = \sqrt{N p q}. \]

Difference of diffusion fluxes was modeled as follows (Fig. 10.18):
\( (d_{2J}) = \frac{N!}{2^N} \left[ \sum_{d=-2J}^{-2J-2(J-1),...} \frac{|d|}{(2J+2d)!} \left(\frac{2J-2d}{2}\right)! \right] + 0 + \sum_{d=2J,4,...}^{2J} \frac{|d|}{(2J+2d)!} \left(\frac{2J-2d}{2}\right)! \]

\[= \frac{N!}{2^N} \left[ \sum_{d=-J-1,...}^{-1} \frac{|2d|}{(2J+2d)!} \left(\frac{2J-2d}{2}\right)! \right] + \sum_{d=1,2,...}^{-1} \frac{|2d|}{(2J+2d)!} \left(\frac{2J-2d}{2}\right)! \]

\[= \frac{N!}{2^{N-2}} \sum_{d=1}^{J} \frac{2d}{(J+d)! (J-d)!} \]

\[= \frac{N!}{2^{N-2}} \sum_{d=1}^{J} \frac{d}{(J+d)! (J-d)!} \]
10.18 Model Discussion

The diffusion process is nothing but movement of solute from high to low concentration, often called as standard Brownian motion, it is an advanced techniques due to its connections with the physical process to derive the overall process of drug absorption, diffusion, metabolism, and excretion. It is one of the best-known stochastic model to extrapolate various diffusion fluxes without loss of any information (Akaike information >reference range). The techniques will also be useful to demonstrate the stochastic process with various stationary-independent increments of plasma concentration with varied time intervals. Since the formulation of model is used to find the explicit solution of simple stochastic differential eqn with a Thomson iteration or Range and Kutta methods and also model will be able to escalate the explicit solution of drug diffusion, time of absorption, time taken for diffusion, and elimination rate by using newer techniques of optimization (Brownian diffusion model). Similarly the blood volume, bioavailability was approximated from the Brownian motion. The model is a more advanced tool because the diffusion with the molecules is not instantaneous, and has certain time interval. During the time absorption is taking place, the change of movement of molecules or drug particles from the region of higher concentration to the region of lower concentration was derived from random walk process (time verifying stochastic). Of course, in addition one has to assume that the motion of the Brownian particles does not create an ordered flow in the surroundings fluid; such a flow would influence the diffusion probability at later times, and in that way act as memory storage that violates the Gaussian assumptions.

10.19 Conclusion

An advanced statistical method is widely used in clinical and medical research to compile the massive data sets that have been collected in clinical trials as well as pharmacokinetic studies. Usually PK intervention is embedded with planning, designing, and decision-making phase. From the induction of drug planning phase to final approval of drug, the statistical modeling plays a major role in taking right decision about drug delivery and manufacturing process. The present chapter briefly describes various advanced statistical modeling approach to pharmacokinetics. These models help the drug manufacturer, stake holders, and clinicians to test the real credibility and acceptability of the new drugs based on numerical simulation techniques, and also our demonstrated models are critical to the researchers for nurturing pharmaceutical and clinical sciences. The clinical practitioners and chemists make use of these models in a better way to approach the new clinical and drug intervention research and also used for new formulation of drug and decisions on patients’ care. The models were effectively beneficial for administrators and policymakers for impacting the health-care system to improve the quality of life of patients and minimize the health costs. The present chapter derives veracity of various advanced analytical and mathematical simulations like Brownian motion and random walk models (constructed based on the optimization techniques), and these recent models will be very useful for the researchers in the establishment of newer clinical and pharmaceutical studies with a varied setup. Finally, our formulated models will be used for the evaluation and classification of drugs in association with plasma concentration and bioavailability. Together, an overall content of the present chapter provides a practical framework with recommendations to guide the development and evaluation of novel regimens to draw a valid conclusive inference about the population or subjects.

References

Ahmed MU et al (2012) Comparative pharmacokinetic and bioequivalence study of azithromycin 500 mg tablet in healthy Bangladeshi volunteers. Int J Clin Pharmacol Ther 50:452–458
Aronow WS, Frishman WH, Cheng-Lai A (2000) Cardiovascular drug therapy in the elderly. Heart Dis 2:151–167
Basavarajaiah DM (2018) Advances in genetic statistics. Educreation Publication, New Delhi, pp 117–120
