ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/biostatic.2017-58451

A Note on Pharmacokinetics Modelling of Theophylline Concentration Data on Patients with Respiratory Diseases

Teofilin Konsantrasyon Verisi ile Solunum Sistemi Hastalığı Olan Hastaların Farmakokinetik Modellemesi Üzerine Bir Not

Isaac Adeola ADENIYI,[a]
Waheed BabatundeYAHYA,[b]
Chinenye Pauline EZENWEKE[a]

[a]Department of Mathematics,
Federal University, Lokoja,
[b]Department of Statistics,
University of Ilorin,
Faculty of Physical Sciences, Ilorin

Received: 11.10.2017
Received in revised form: 28.12.2017
Accepted: 29.12.2017
Available online: 05.04.2018

Correspondence:
Waheed Babatunde YAHYA
University of Ilorin,
Faculty of Physical Sciences, Ilorin,
Department of Statistics,
NIGERIA/NİJERYA
wbyahya@unilorin.edu.ng

ABSTRACT Pharmacokinetics which describes the time course of drug absorption, distribution, metabolism, and excretion in the body is critical in formulating drug therapy. Nonlinear Mixed Effects (NLME) models are popularly used in many longitudinal studies, including human immunodeficiency viral dynamics, pharmacokinetic analyses, and studies of growth and decay.This work aimed to develop efficient NLME models for analyzing Theophylline concentration data within the pharmacokinetics framework. The data consisted of Theophylline concentration (mg/L) measurements of 12 asthmatic patients who were treated with oral Theophylline. The serum concentrations were measured at 11 times per subject over 25 hours periods. Hence, a total of 132 observations were obtained. Six different pharmacokinetics models were fitted in a step-wise manner to these data within the framework of NLME techniques. The best of these models that yielded the most efficient estimates of the physiological factors such as absorption rate (k_a), elimination rate (k_e), and clearance (C_l) in the patients was determined using suitable model selection criteria. The results showed that the clearance and absorption rate have mixed effects with estimated values of $\hat{C}_l = 0.0397$ and $\hat{k}_a$= 1.54203 (for fixed effects) while the effect of the elimination rate in all the patients is fixed with the estimated value of $\hat{k}_e$ =0.0860. Also, the low estimated standard deviations of the random effects components of C_l(0.1699) and k_a(0.6384)over the entire samples is a clear evidence that the fitted model was quite consistent and efficient.Results from this study would further serve as useful guides to clinicians and drug developers in the proper formulation and administration of Theophylline therapy on patients suffering from respiratory diseases.

Keywords: Pharmacokinetics; theophylline concentration; nonlinear mixed effects model; compartmental model

ÖZET Vücutta zamanla meydana gelen ilaç emilimi, dağılımı, metabolizması ve boşaltımını tanımlayan farmakokinetikler, ilaç terapi formüllerinde çok önemlidir. Doğrusal olmayan karışık etki (DOKE) modelleri, büyüme ve çürüme çalışmaları, farmakokinetik analizler ve insan immün yetmezliği viral dinamiklerini kapsayan birçok longitudinal çalışmada popüler olarak kullanılmaktadır. Bu çalışmada farmakokinetik yapı için teofilin konsantrasyon verisi analiz edilerek etkili DOKE modelleri geliştirmek amaçlanmaktadır. Veri seti, ağızdan teofilin ile tedavi edilen 12 astım hastasının teofilin konsantrasyon ölçümlerini içermektedir. Serum konsantrasyonları, 25 saatlik periyotlarla her hastadan 11 kez ölçülmüştür. Dolayısıyla 132 gözlem elde edilmiştir. DOKE teknikleri çerçevesinde bu veri seti için altı farklı farmakokinetik model aşamalı olarak tahmin edilmiştir. Hastalarda emilim hızı (k_a), eleme hızı (k_e) ve aralık (C_l) gibi fizyolojik faktörlerin en etkili tahminini sağlayan en iyi modeller uygun model seçim kriteri kullanılarak belirlenmiştir. Sonuçlar, eleme hızının tüm hastalarda $\hat{k}_e = 0.0860$ tahmin değeri ile sabit olmasına rağmen, aralık ve emilim hızının $C_l = 0.0397$ ve $\hat{k}_a = 1.54203$ (sabit etkiler için) tahmin değerleri ile karışık etkiye sahip olduğunu göstermiştir. Ayrıca, tüm örneklemlerden elde edilen C_l(0.1699) ve k_a(0.6384) rasgele etki bileşenlerinin standart sapmasının düşük tahmin edilmesi, tahmin edilen modelin tutarlı ve etkili olduğunu gösteren açık bir kanıttır. Bu çalışmanın sonuçları klinisyenlere ve ilaç geliştiricilere solunum yolu hastalığı ile savaşan hastaların teofilin terapisinin yönetimi ve formülasyonunda yararlı bir rehber olarak hizmet edecektir.

Anahtar Kelimeler: Farmakokinetikler; teofilin konsantrasyonu; doğrusal olmayan karışık etki modeli; bölümlü model

One of the most common studies where clustered data arise is in pharmacokinetic (PK) analysis in which serial blood samples are collected from each of several subjects following doses of a drug and assayed for drug concentration. [1] Pharmacokinetic analysis is important in developing dosing strategies for different subgroups.[2] Population pharmacokinetic analysis provides a very valuable aid to the development process of drug dosing strategies by identifying and quantifying the variability in drug concentrations in the body.[3] 'Population' here clarifies the fact that one wish to gain an understanding of the dose/concentration relationship across different possible groups (i.e. population) as defined by covariates such as sex, age and weight.

There are different statistical approaches to population pharmacokinetic data analysis which include the Two-stage, naive pooled data, and Non-linear Mixed Effects (NLME) modelling approaches among others.[4-6] The literature has however favoured the use of NLME method for modelling pharmacokinetic data more than the naive pooled data and two-stage approaches due to its flexibility, applicability to sparse data and ability to provide less biased estimates of the between-individual variation.[2]

The NLME models constitute powerful tools not only for estimating the population pharmacokinetic parameters but as well as the between-individual variance parameters.[7] The appropriateness of the assumptions contained in these models may sometimes be difficult to validate, especially those concerning the unobservable quantities. It is therefore important that medical/pharmacological information is used during modeling.[7]

Given the above background, the present work aimed to investigate the use of NLME modelling techniques for the analysis of complex pharmacokinetic data. The application of this modelling technique was illustrated with real life data set on theophylline concentration pharmacokinetics.

The subsequent sections of this paper are arranged as follows. The general concept of NLME models was introduced in Section 2. Section 3 provided the basic concepts of pharmacokinetics while Section 4 presented the description of theophylline concentration data set used and the compartmental pharmacokinetic models fitted to the data. The estimation procedure, analysis and results were presented in Section 5 while detailed discussion of results and conclusion were given in Section 6.

NONLINEAR MIXED EFFECT MODELS

The NLME models constitute powerful tools for handling clustered data like the pharmacokinetic data discussed here.[8] They are especially useful to account for inter-subject and intra-subject variations which is crucial for developing dosage strategies for drugs especially those with a narrow therapeutic range, therefore facilitating adaptation of drug dosage to the individual patient (therapeutic drug monitoring)[2].In these models, the response is assumed to be a function of fixed (population) effects, non-observable individual specific random effects, and an error term. Also, some of the fixed and/or random effects occur nonlinearly in the response function.[8]

Mixed-effects models are primarily used to describe relationships between a response variable and some covariates in data that are grouped according to one or more classification factors.[9] The most general form of NLME models as given by Lindstrom and Bates[6] is of the form;

$$y_{ij} = f(\boldsymbol{\Phi}_i, \boldsymbol{X}_{ij}) + \epsilon_{ij}, i = 1, \dots, m, \; j = 1, \dots, n_i, \quad (1)$$

where m is the number of groups or subjects, n_i is the number of observations in the i^{th} group or subject, y_{ij} is the j^{th} response for the i^{th}group or subject, f is a general nonlinear function, $\boldsymbol{X}_{ij}$ is the

predictor vector for the j^{th} response of the i^{th} group or subject, the residual errors $\epsilon_{ij}$are assumed to be independent and identically distributed normal random variables with mean zero and variance σ^2. The quantity $\boldsymbol{\Phi}_i$ is a mixed effects parameter vector that is expressed as a linear function of the fixed effects vector $\boldsymbol{\beta}$ and the random effects vector $\boldsymbol{b}_i$ and is given by the equation;

$$\boldsymbol{\Phi}_i = \boldsymbol{A}_i\boldsymbol{\beta} + \boldsymbol{B}_i\boldsymbol{b}_i \quad (2)$$

where $\boldsymbol{A}_i$ and $\boldsymbol{B}_i$, which might depend on the fixed and/or random effects terms and possibly some covariates are the design matrices for the fixed effects vector β and random effects vector $\boldsymbol{b}_i$ respectively. It is assumed that $\boldsymbol{b}_i$ is multivariate normally distributed with mean vector zero and variance-covariance matrix $\boldsymbol{D}$ and is independent of ϵ_{ij} for all i and j.

The parameters in a mixed effects model can be classified into two types: fixed effects, associated with the average effect of predictors on the response, and variance-covariance components, associated with the covariance structure of the random effects and of the error term.[7] In many practical applications estimates of the random effects are also of interest.[7] There are typically no analytic or closed-form expressions for parameter estimates since the likelihoods involve integrations with respect to the unobservable random effects.[8] Therefore estimation relies on approximation. Several approximations have been proposed for NLME models. Some of them include alternating algorithm proposed by Lindstrom and Bates, Laplacian approximation, importance sampling, Gaussian quadrature and full Exponential Laplace approximation.[6,10-13] Using any of the methods listed above requires an iterative algorithm which requires the use of computer programs because computation is very intensive. There is still an on-going debate in the statistical literature about estimation methods for NLME models even after so many research works have been carried out concerning this over the years. Pinheiro found out that, in most cases, Lindstrom and Bates approximation gives very accurate results and is computational efficient.[6,7]

NLME modeling is a rich class models which is applicable in many fields.[14] Model building techniques for NLME models involve determining which effects should have an associated random component and which should be purely fixed; using covariates to explain inter-individual parameter variability; using structured random effects variance-covariance matrices (e.g. diagonal matrices) to reduce the number of parameters in the model.[7]

The focus of the present work is on the application of NLME modeling technique to the analysis of pharmacokinetic data.The model building technique is iterative in nature which involves fitting a tentative model that is modified after a number of re-fittings (iterations) to arrive at the best model. The Akaike Information Criterion (AIC), the Bayesian Criterion (BIC), and the likelihood ratio test (LRT) can be used to discriminate between models.[9,15-17] The process is repeated until no further improvement is possible. In comparing alternative models one must also analyze the residuals from the fit, checking for departures from the assumptions in the model.[7]

Without loss of generality, a heuristic algorithm for NLME modelling technique to model a pharmacokinetic dataset include the following five basic steps: i.) plot the structure of data; ii.) fit model separately for each group; iii.) fit non-linear mixed effect model; iv.) analyse non-linear mixed effect model; and v.) incorporate covariates into the model if necessary.

It is important to remark that, the basic NLME model can be extended by allowing the variability of the residual errors to have a different structure apart from the homoscedastic structure.[18] Such classes of variance

models for modeling heteroscedasticity include "*fixed variance*", "*different variances per stratum*", "*power of a variance covariate*" and "*constant plus power of a variance covariate*" amongst others. However, following Davidian and Giltinan, the general variance function for the residual errors is defined as[19]

$$Var\,(\epsilon_{ij} \mid \boldsymbol{b_i}) = \sigma^2 g^2(\mu_{ij}, \boldsymbol{v}_{ij}, \boldsymbol{\delta}),\ i = 1,\ldots,m,\ j = 1,\ldots,n_i, \quad (3)$$

where $\mu_{ij} = E\,[y_{ij}|\boldsymbol{b_i}]$, $\boldsymbol{v}_{ij}$ is a vector of variance-covariates,$\boldsymbol{\delta}$is a vector of variance parameters and $g(\cdot)$ is the variance function, assumed continuous in $\boldsymbol{\delta}$.The variance structures of the residual errors of most PK data belong to the class of "*power of a variance covariate*" where the covariate is the fitted concentration. But when the range of possible values of the observations contains zero, it is better to model the errors using a combined additive (homoscedastic)and proportional error model which is the "*constant plus power of a variance covariate*" class.[20] Therefore, following Davidian and Giltinan, the *constant plus power of a variance* model as employed in this work is of the form[19]

$$Var\,(\epsilon_{ij}) = \sigma^2 g^2(\boldsymbol{v}_{ij}, \boldsymbol{\delta}) \quad (4)$$

where

$$g(\boldsymbol{v}_{ij}, \boldsymbol{\delta}) = \delta_1 + |\boldsymbol{v}_{ij}|^{\delta_2} \quad (5)$$

It is worth to remark that, the analysis carried out in this work is based on the alternating algorithm of Lindstrom and Bates (1990) which was later developed into statistical package for NLME modelling by Pinheiro et al. and documented under 'nlme' package in R software (www.R-project.org).[21] Thus, all the analytical tasks performed in this work were carried out using the R statistical package.

PHARMACOKINETICS CONCEPTS

Pharmacokinetics as defined earlier is the study of the mechanisms and kinetics of drugs' absorption, distribution, metabolism, and elimination by the body in animals or humans.[22] Gibaldi and Perrier defined Pharmacokinetics as the study of the time course of drug absorption, distribution, metabolism, and excretion. In simple terms, Pharmacokinetics is the study of what the body does to the drug and this is important in developing dosing strategies.[23,24]

A drug's effect is often related to its concentration at the site of action, so it would be useful to monitor this concentration in animals (rats) or selected humans having the ailment for which the drug is designed.[25] This leads to expressing the drug concentration mathematically in terms of some biological factors referred to as parameters. These expressions are called pharmacokinetic models.

To facilitate complete understanding of the distribution, elimination, activity, and toxicity of a drug, it is very important to have a complete detailed description of the simplest pharmacokinetic model consistent with the data observed for the drug tendency in man.[25] This becomes increasingly important with drugs that have a low therapeutic index and serious side-effects such as the cardiac glycoside digoxin.[26]

Pharmacokinetic data analysis can be compartmental or non-compartmental.[2] The Non-Compartmental Analysis (NCA) which is not commonly used is simple and does not require the assumption of a specific compartmental model for the drug of interest. However, the relatively large number of data points required for a reliable estimation of pharmacokinetic parameters using the NCA method may be disadvantageous.[2] The compartmental model on the other hand requires that the body be represented by one or more compartments and hence, pharmacokinetics of drugs is generally represented by compartmental models. The model could have one, two or multi-compartments.

In this paper, we restrict ourselves to single-compartment pharmacokinetic models. Most of these models are structured such that the relationship between the drug concentration and the parameters are nonlinear and as a result modeling becomes very tasking.

One procedure for estimating the parameters of pharmacokinetic model is the Standard Two-Stage (STS) approach which requires fitting a pharmacokinetic model to the data of each individual. Thereafter, summary statistics are computed for the total collection of individual parameter estimates.[4] Following this approach, the between-individual variation tends to beover-estimated while it might not be efficient to apply this approach for individual model fit when the individual data are too sparse.[2,27]

Another procedure for compartmental modeling is the non-linear mixed effects (NLME) modeling technique which has been described in Section 2. This procedure, as adopted in this study, constitutes powerful tools not only for estimating the population pharmacokinetic parameters but as well as the between-individual variance parameters.[7] The clustered nature of pharmacokinetic data makes it a good candidate for NLME models.

RELEVANT PHARMACOKINETIC PARAMETERS

According to Younggil, the following are some of the necessary pharmacokinetic parameters that should be determined during development of dosage strategies for a drug: i.) Total clearance (Cl) ii.) Fraction of dose excreted unchanged in urine (f_e) iii. Volume of distribution at steady state (V_{ss}) iv. Volume of distribution during the terminal phase (V_z) v. Blood/plasma concentration ratio vi. Terminal half-life ($t_{1/2,z}$) vii. Fraction of unbound drug in plasma (f_u) viii. Elimination rate (k_e) ix. Area under curve (AUC) x. Bioavailable fraction of dose (F), if applicable, and xi. Absorption rate constant (k_a), if applicable.[28]

It is important to note that it may not be necessary or possible to obtain all the parameters listed above for some drugs before one can fully describe the pharmacokinetics of the drugs.[2]

MODELLING THEOPHYLLINE PHARMACOKINETIC DATA

Theophylline (also known as 1,3-dimethylxanthine) is a Bronchodilator which is a drug used in therapy for respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD).[29] For Theophylline, a one-compartment model is the standard[7]and is described below.

Suppose that a (drug) substance enters the body via ingestion, the first-order one-compartment open PK model that describes the working of the substance in the body is defined by the following system of Ordinary Differential Equations (ODEs):[30]

$$\dot{y}_1(t) = -k_a y_1(t) \tag{6}$$

$$\dot{y}_2(t) = k_a y_1(t) - k_e y_2(t), \qquad k_a,\ k_e > 0 \tag{7}$$

where $\dot{y}_1(t)$ represents the mass of drug present at time t in the external compartment (stomach/intestines), $\dot{y}_2(t)$ is the mass of drug absorbed into the body (e.g. plasma). The coefficient k_a describes the absorption rate from the external to the central compartment while k_e is the elimination rate from the central compartment.$\dot{y}_1(t)$ and $\dot{y}_2(t)$shall henceforth be referred to as just $\dot{y}_1$ and $\dot{y}_2$ respectively for simplicity.

If we differentiate (4) again we have,

$$\ddot{y}_2 = k_a \dot{y}_1 - k_e \dot{y}_2 \tag{8}$$

If we substitute (6) into (7) for $\dot{y}_1$ and the resulting equation in term of $\dot{y}_1$ is again substituted into (8), we shall have a single second-order differential equation for the central compartment of the form;

$$\ddot{y}_2 + (k_a + k_e)\dot{y}_2 + k_a k_e y_2 = 0 \quad (9)$$

Solving (9) above results to the quadratic equation below

$$\alpha^2 + (k_a + k_e)\alpha + k_a k_e = 0 \quad (10)$$

The solutions to (10) are;$\alpha_1 = -k_e$and $\alpha_2 = -k_a$. Therefore, a solution for (6) is

$$y_2(t) = K_1 exp\{\alpha_1 t\} + K_2 exp\{\alpha_2 t\}$$

$$\rightarrow y_2(t) = K_1 exp\{-k_e t\} + K_2 exp\{-k_a t\} \quad (11)$$

Putting (11) along with its first derivative into (7) leads to the general solution for y_1. The general solution of the system consisting of equations (6) and (7) therefore is:

$$y_1(t) = \frac{k_e - k_a}{k_a} K_2 exp\{-k_a t\} \quad (12)$$

$$y_2(t) = K_1 exp\{-k_e t\} + K_2 exp\{-k_a t\} \quad (13)$$

Now let $y_1(t_d)$ be the mass of the drug in the body at time (t_d) when the drug was ingested, therefore $y_1(t_d) = D$ (dose ingested) and $y_2(t_d) = 0$ (because no drug would have been absorbed yet). Solving (12) and (13) simultaneously with $y_1(t_d) = D$ and $y_2(t_d) = 0$ leads to

$$K_2 = \frac{D k_a}{k_e - k_a} exp\{k_a t_d\}$$

$$K_1 = \frac{D k_a}{k_e - k_a} exp\{k_e t_d\}$$

Putting the solutions for K_1 and K_2 in (12) and (13), the explicit solutions for (6) and (7) become

$$y_1(t) = D exp\{-k_a(t - t_d)\} \quad (14)$$

$$y_2(t) = \frac{D k_a}{k_a - k_e} exp\{-k_e(t - t_d)\} - exp\{-k_a(t - t_d)\} \quad (15)$$

Dividing equation (15) by the volume of distribution V to have the expression to be for concentration gives

$$C_t = \frac{D k_a}{V(k_a - k_e)} (exp\{-k_e(t - t_d)\} - exp\{-k_a(t - t_d)\})$$

But since $V = \frac{C_l}{k_e}$, so we have

$$C_t = \frac{D k_e k_a}{C_l(k_a - k_e)} (exp\{-k_e(t - t_d)\} - exp\{-k_a(t - t_d)\})$$

where C_l is the clearance. In most cases, t_d is taken to be equal to zero (i.e., $t_d = 0$), the model therefore can be simply written as

$$C_t = \frac{D k_e k_a}{C_l(k_a - k_e)} (exp\{-k_e t\} - exp\{-k_a t\}) \quad (16)$$

where C_t is the plasma concentration at time t (mg/L), t is the time (hr), D is the dose (mg/kg), C_l is the clearance (L/kg), k_e is the elimination rate constant (1/hr), and k_a is the absorption rate constant (1/hr).

When estimating model (16) using the NLME modeling techniques, it is necessary to take the logarithms of the rates (k_e, k_a and C_l) to ensure that the rates are positive. Therefore, model (16) can thus be written as

$$C_t = \frac{Dexp\{lk_e + lk_a - lC_l\}}{exp\{lk_a\} - exp\{lk_e\}}(exp\{-exp\{lk_e\}t\} - exp\{-exp\{lk_a\}t\}) \quad (17)$$

where $lC_l = \log(C_l)$, $lk_a = \log(k_a)$, and $lk_e = \log(k_e)$. The required NLME model can thus be written as

$$C_{ij} = \frac{D_i exp\{\phi_{3i} + \phi_{2i} - \phi_{1i}\}}{exp\{\phi_{2i}\} - exp\{\phi_{3i}\}} \times \left(exp\left[-exp\{\phi_{3i}\}t_{ij}\right] - exp\left[-exp\{\phi_{2i}t_{ij}\}\right]\right) + \epsilon_{ij} \quad (18)$$

The C_{ij} in model (18) represents Y_i of model (6) which denotes the drug concentration in the blood. Hence, the full description of (18) within the NLME model framework is as follows;

$$y_{ij} = C_{ij} = f\ (\boldsymbol{\Phi}_i, [t_{ij}, D_i])\ + \epsilon \quad (19)$$

where $\boldsymbol{\Phi}_i = \boldsymbol{A}_i\boldsymbol{\beta} + \boldsymbol{B}_i\boldsymbol{b}_i$ as defined in (2) with

$$\boldsymbol{A}_i = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}; \boldsymbol{B}_i = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

and

$$\boldsymbol{\Phi}_i = \begin{bmatrix} lC_{l_i} \\ lk_{a_i} \\ lk_{e_i} \end{bmatrix} = \begin{bmatrix} \Phi_{1i} \\ \Phi_{2i} \\ \Phi_{3i} \end{bmatrix} = \boldsymbol{A}_i \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} + \boldsymbol{B}_i \begin{bmatrix} \mathrm{b}_{1i} \\ \mathrm{b}_{2i} \\ \mathrm{b}_{3i} \end{bmatrix}$$

$$\rightarrow \boldsymbol{\Phi}_i = \begin{bmatrix} \Phi_{1i} \\ \Phi_{2i} \\ \Phi_{3i} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \mathrm{b}_{1i} \\ \mathrm{b}_{2i} \\ \mathrm{b}_{3i} \end{bmatrix}$$

Therefore, model (18) becomes

$$C_{ij} = \frac{D_i exp\{(\beta_3 + b_{3i}) + (\beta_2 + b_{2i}) - (\beta_1 + b_{1i})\}}{exp\{(\beta_2 + b_{2i})\} - exp\{(\beta_3 + b_{3i})\}}\left(exp\left[-exp\{(\beta_3 + b_{3i})\}t_{ij}\right] - exp\left[-exp\{(\beta_2 + b_{2i})\}t_{ij}\right]\right) + \epsilon_{ij} \quad (20)$$

with $i = 1, \ldots, m$ subjects and $j = 1, \ldots, n_i$ measurements, C_{ij} is the j^{th}measured plasma concentration for the i^{th} subject, t_{ij} is the time at which the j^{th} measurement for the i^{th}subject is taken.

It should be noted that not all the terms in the model may eventually be present after the analysis of a given data. Also, the use of covariates in modelling also changes the structure of $\boldsymbol{\Phi}$ in the model. For example, if a covariate like weight is found to be linearly related with the absorption rate lk_a, then the structure of $\boldsymbol{\Phi}_i$above changes to

$$\boldsymbol{\Phi}_i = \begin{bmatrix} \Phi_{1i} \\ \Phi_{2i} \\ \Phi_{3i} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & Wt_i \\ 0 & 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_4 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \mathrm{b}_{1i} \\ \mathrm{b}_{2i} \\ \mathrm{b}_{3i} \end{bmatrix}$$

which incorporates the weight variable Wt_i of the i^{th} subject while $\boldsymbol{\beta}_4$ is the fixed effect parameter of weight on lk_a. The variation in $\boldsymbol{\Phi}_{2i}$ may be explained straight away by Wt_i, hence b_{2i} may be deleted from the model without any loss in efficiency.

DATA DESCRIPTION

The data employed in this study were published data discussed and used by Boeckmann et al.[31]. The data consist of Theophylline concentration (mg/L) measurements on 12 asthmatic patients who were treated

with oral Theophylline. The serum concentrations were measured at 11 times per subject over 25 hours period, hence a total of 132 observations were obtained. The data contained different doses of oral Theophylline administered to each patient, the Theophylline concentration, weight and concentration time (in hours).The doses were adjusted for weight and ranged from 3.10 mg to 5.86 mg, while the concentration measurements ranged from 0.00 mg/L to 11.4 mg/L with mean concentration of 4.96 mg/L and standard deviation of 2.87 mg/L. These measurements were taken within a range of 0hr to 24.65 hr after dose. Initial body weights ranged from 54.6 kg to 86.4 kg with a mean of 69.58 kg and standard deviation of 9.13 kg. The detailed summary statistics of the clinical characteristics in the data are presented in Table 1.

TABLE 1: The summary statistics of the clinical characteristics of all the 12 asthmatic patients who were treated with oral Theophylline. The serum concentration in each patient was measured 11 times within 25 hours period.

	Mean	Median	Minimum	Maximum	Std. dev.
Oral Dose (mg/kg)	4.63	4.53	3.10	5.86	0.72
Weight (kg)	69.58	70.50	54.60	86.40	9.13
Time (hr)	5.89	3.53	0.00	24.65	6.93
Theophylline Concentration(mg/L)	4.96	5.28	0.00	11.40	2.87

ANALYSIS AND RESULTS

The NLME models were fitted to the Theophylline data described above based on the models' formulations in Section 4. The "nlme" package[21] in R statistical software[32] was employed for fitting the NLME models.

As a preliminary step, it is important to determine which of the rates (k_e, k_a and C_l to be estimated) should be fixed, random or mixed. Figure 1 is the plot of serum concentration for each subject in the study against time. All the graphs in Figure 1 suggested that the serum concentration of the asthma patients depicted non-linear structure with time. As a result, we fitted separate nonlinear regression model on the serum concentration observed on each patient. This enables us to detect between-subject variation through the plots of the 95% confidence intervals for each of the rates.

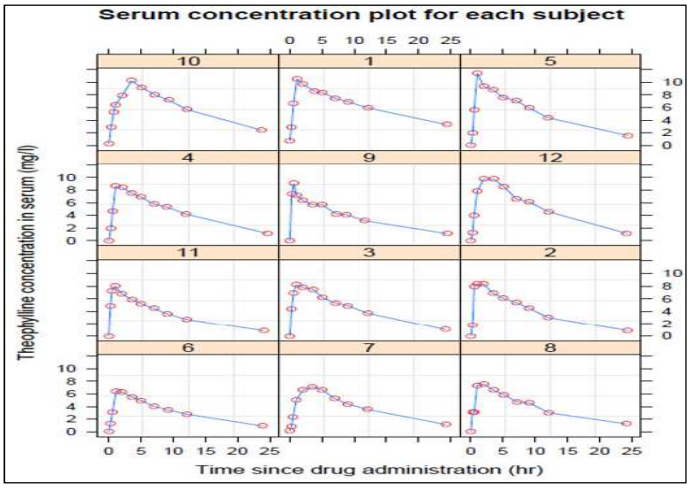


FIGURE 1: Plots of serum concentration against time for all subjects.

As can be observed in Figure 2, the plots of the individual estimated 95% confidence interval suggested that the elimination rate (k_e) can be considered fixed since it seems to be characterized with little variation among subjects while the between-subject variations of the estimates of both the absorption rate (k_a) and clearance (C_l) are visibly high. Hence, both k_a and C_l can be considered to contain both the fixed and random effects.

For a clear overview of the relationship between the three estimated rates, we present the pair-wise scatter plots of the individual nonlinear least squares estimates of the rates as shown in Figure 3 where it can be observed that the first subject in the sample seems to have a very low k_a and k_e values while the ninth subject has a high k_a value. Not only that, the linear relationship between the elimination rate (k_e) and clearance (C_l) is also apparent from the pair-wise plot in Figure 4 which simply underscores the co-movement of the elimination rate and the clearance in theophylline drug administration.

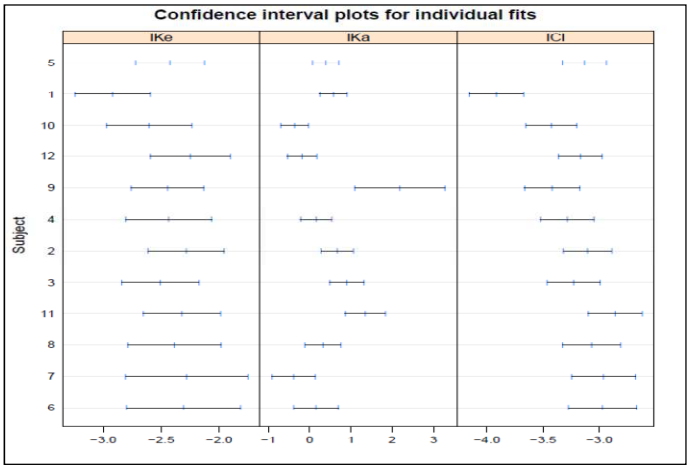


FIGURE 2: Plots of individual 95% confidence intervals of the fitted serum concentrations for all subjects.

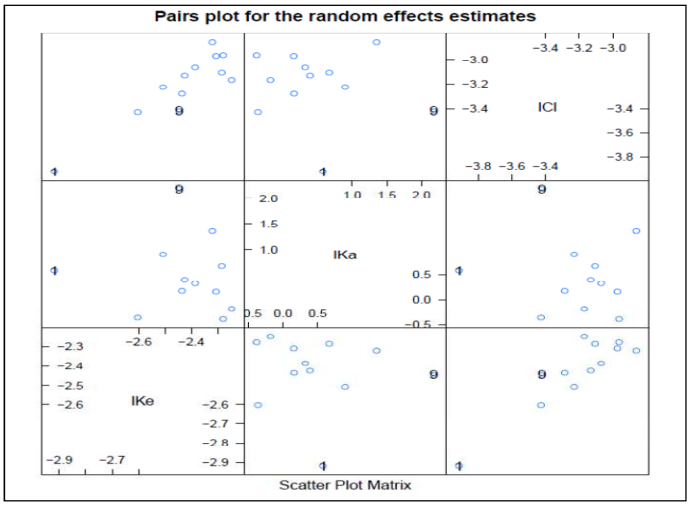


FIGURE 3: Pairs plots of random effects of the parameters of the pharmacokinetic model.

Using the operational model in (20) with all its components remained as defined, the NLME model was fitted to the theophylline data as presented in the Appendix in which all the rates were considered fully mixed. This model, as given below is called **model 1**

$$C_{ij} = \frac{D_i exp\{(\beta_3 + b_{3i}) + (\beta_2 + b_{2i}) - (\beta_1 + b_{1i})\}}{exp\{(\beta_2 + b_{2i})\} - exp\{(\beta_3 + b_{3i})\}} \big(exp\big[-exp\{(\beta_3 + b_{3i})\}t_{ij}\big] - exp\big[-exp\{(\beta_2 + b_{2i})\}t_{ij}\big]\big) + \epsilon_{ij}$$

with $i = 1, \ldots, 12$ patients or groups and $j = 1, \ldots, 11$ measurements per patient or group, C_{ij} is the j^{th} measured plasma concentration for the i^{th} patient, t_{ij} is the time at which the j^{th} measurement for the i^{th} patient is taken. The results of the fitted model are presented in Table 2 in which the estimates of the fixed effects parameters and the standard deviations of the random effects parameters of the model are reported.

TABLE 2: Estimates of the parameters in all the fitted models. NB: The fixed effects of $\boldsymbol{lC_l}$, $\boldsymbol{lk_a}$, $\boldsymbol{lk_e}$ were estimated as β_1, β_2 and β_3 respectively while the standard deviations of their corresponding random effects over all the subjects were estimated as sd_1, sd_2 and sd_3 respectively.

Model	Rates	Fixed effect Parameters $\beta_{k,} k = 1,2,3$	Standard Deviation of Random effects $sd_{k,} k = 1,2,3$	Residual errors variability parameters	
				$\boldsymbol{\delta_1}$	$\boldsymbol{\delta_2}$
Model 1	$\boldsymbol{lk_a}$	0.4514	0.6378	-	-
	$\boldsymbol{lk_e}$	-2.4327	0.1311		
	$\boldsymbol{lC_l}$	-3.2145	0.2512		
Model 2	$\boldsymbol{lk_a}$	0.4655	0.6439	-	-
	$\boldsymbol{lk_e}$	-2.4547	1.9308x10^{-5}		
	$\boldsymbol{lC_l}$	-3.2272	0.1669		
Model 3	$\boldsymbol{lk_a}$	0.4657	0.6436	-	-
	$\boldsymbol{lk_e}$	-2.4547	-		
	$\boldsymbol{lC_l}$	-3.2272	0.1669		
Model 4	$\boldsymbol{lk_a}$	0.4331	0.6384	0.7293	0.3191
	$\boldsymbol{lk_e}$	-2.4538	-		
	$\boldsymbol{lC_l}$	-3.2275	0.1699		
Model 5	$\boldsymbol{lk_a}$	0.4334	0.6334	0.7251	0.3164
	$\boldsymbol{lk_e}$	-2.4560	-		
	$\boldsymbol{lC_l}$	-2.7104	0.1551		
	$\boldsymbol{\beta_4}$	-0.0075	-		
Model 6	$\boldsymbol{lk_a}$	-3.5491	0.0841	0.2851	0.0887
	$\boldsymbol{lk_e}$	0.4310	-		
	$\boldsymbol{lC_l}$	-3.2577	0.1359		
	$\boldsymbol{\beta_4}$	0.0149	-		

Recall that, in the formulation of the PK model, it is assumed that k_a, k_e and C_l are purely independent. However, the correlations of their estimated logarithm values as presented in Table 3 indicated a high correlation between lk_e and lC_l ($\hat{\rho} = 0.995$, $p < 0.001$), which simply suggests interdependency between these two PK parameters. As a result, we fitted another model such that the estimated random effects in

the model are independent and that the random-effects variance-covariance structure is not ill-conditioned. This second model is labeled **model 2** in Table 2. In essence, the regression equation for **model 2** is the same as that of **model 1**, the exception is that the variance-covariance matrix of the error term in **model 2** is not ill-conditioned which guarantees independence of random effects parameters. The fixed effects and the standard deviations of the random effects obtained for the second fitted model (**model 2**) are presented in Table 2.

TABLE 3: Correlation values between the estimated random effects terms in Model 1.

	lk_a	lk_e	lC_l
lk_a	1	0.012	-0.089
lk_e	-	1	0.995
lC_l	-	-	1

TABLE 4: Comparison and choice of models using the BIC and LRT.

Model	BIC	Log-likelihood	Number of parameters	P-value	Winning model
Model 1	395.4702	-173.3211	10		
Model 2	388.2266	-177.0235	7	0.0601 (Model 1 vs model 2)	Model 2
Model 3	383.3397	-177.0214	6	0.9488 (Model 2 vs model 3)	Model 3
Model 4	**374.4193**	-167.6784	8	<0.0001 (Model 3 vs model 4)	Model 4
Model 5	377.4424	-166.7486	9	0.1727 (Model 6 vs model 4)	Model 4
Model 6	440.7853	-198.4200	9	<0.0001(Model 6 vs model 4)	Model 4

For ease of models` comparison, the values of the BIC[33-35] and the Likelihood Ratio Test (LRT)[36] for the fitted models were equally reported in Table 4. From the results in Table 4, it can be observed that both the BIC and the p-values of the LRT indicated that the simpler **model 2** cannot be said to be poorer than **model 1**, in fact, the BIC suggests that **model 2** is better.

It could be observed from the results of **model 2** in Table 2 that the standard deviation of the estimated parameter lk_e(considered random) is grossly smaller than those of the other two parameters. This further suggested that the effects of lk_e should be considered fixed. As a result, we proceeded to fit another model (**model 3**) where lk_e is considered purely fixed and a comparison of **model 3** with **model 2** is provided in Table 4. The value of BIC for **model 3** which is relatively smaller than that of **model 2** suggested that the simpler model, **model 3**with fixed effect parameter $lk_e$was a better model than **model 2**.The regression equation for **model 3** is a reduced version of model (20) for **model 1** and is given by

$$C_{ij} = \left[\frac{Dexp\{(\beta_3)+(\beta_2+b_{2i})-(\beta_1+b_{1i})\}}{exp\{(\beta_2+b_{2i})\}-exp\{(\beta_3)\}} \times \left(exp\left[-exp\{(\beta_3)\}t_{ij}\right] - exp\left[-exp\{(\beta_2+b_{2i})\}t_{ij}\right]\right)\right] + \epsilon_{ij} \quad (21)$$

As a way to check for the adequacy of the fitted **model 3**, the plot of standardized residuals against the fitted values of plasma concentration is presented by Figure 4a from where it can be observed that the error variance increases as the fitted Theophylline concentration increases. This therefore suggested that a homoscedastic variance model for the within-group residuals cannot be assumed for the data. Hence, a heteroscedastic model where the errors are represented by a combined

additive (homoscedastic) and proportional error model need to be fitted using the model`s variance formulations in equations (4) and (5). A new model with these properties was fitted and labeled **model 4** the results of which are again reported in Table 2. The regression equation for **model 4** is the same as that of **model 3**. The only difference between **model 3** and **model 4** is in their error variance structures which in turn caused a slight difference in the values of the main parameter estimates. The main parameter estimates here are the fixed effects parameters and random effects covariance components.

A comparison of **model 4** with the previously fitted model, **model 3** using the BIC criteria indicated that **model 4** with BIC value of 374.4193 is a better model than **model 3** with a relatively higher BIC value of 383.3397 as shown in Table 4. We equally provide the plot of the standardized residuals of **model 4** versus the fitted values as shown by Figure 4b from where it can be observed thatthe error variance increases as the fitted Theophylline concentration increases which simply confirms the adequacy of **model 4** as an heteroscedastic NLME model.

Furthermore, it is imperative to examine the possible impacts of patients` weights (if any) on inter-individual variations of some of the PK parameters especially, the absorption rate $k_a$and the clearance $C_l$ whose effects are considered to be random. Thus, we investigated if patients` weights account for inter-individual variation in PK parameters k_a and C_l. To this effect, we obtain the scatter plot of the weight covariate versus the estimated absorption rate $\hat{k}_a$ and clearance $\hat{C}_l$ as determined from **model 4**. This scatter plots are shown in Figure 5. The scatter plots in Figure 6 seem to indicate increase in absorption rate of theophylline as patient's weight increases while the clearance decreases as weight increases. The NLME model fitted with weight variable (Wt_i) included as covariate with each of the PK parameters $lC_l$and lk_a are labelled **model 5** and **model 6** respectively the results of which are presented in Table 2. The results of BIC and LRT for comparing **models 5** and **6** each with **model 4** are presented in Table 4. The regression equations for **model 5** and **model 6** with weight variable Wt_i included as covariate in place of random effect components of the PK parameters lC_l (clearance) and lk_a (absorption rate) are given by

$$C_{ij} = \left[\frac{Dexp\{(\beta_3)+(\beta_2+b_{2i})-(\beta_1+Wt_i\beta_4)\}}{exp\{(\beta_2+b_{2i})\}-exp\{(\beta_3)\}}\left(exp\left[-exp\{(\beta_3)\}t_{ij}\right] - exp\left[-exp\{(\beta_2+b_{2i})\}t_{ij}\right]\right)\right] + \epsilon_{ij} \quad (22)$$

and

$$C_{ij} = \left[\frac{Dexp\{(\beta_3)+(\beta_2+Wt_i\beta_4)-(\beta_1+b_{1i})\}}{exp\{(\beta_2+Wt_i\beta_4)\}-exp\{(\beta_3)\}}\left(exp\left[-exp\{(\beta_3)\}t_{ij}\right] - exp\left[-exp\{(\beta_2+Wt_i\beta_4)\}t_{ij}\right]\right)\right] + \epsilon_{ij} \quad (23)$$

respectively, where Wt_i is the weight of the i^{th} patient.

A comparison of each **models 5** and **6** with **model 4** using the BIC criteria as shown in Table 4 again indicated that parsimonious **model 4** is better than both **models 5** and **6**. This simply showed that the inclusion of the weight variable as covariate in the PK model is not necessary, more so that the covariate (D) that represents the amount of oral dose of theophylline administered on the patients in the model is weight adjusted. Hence, **model 4** becomes the final chosen model, and the PK model building process for the theophylline data hereby terminates.

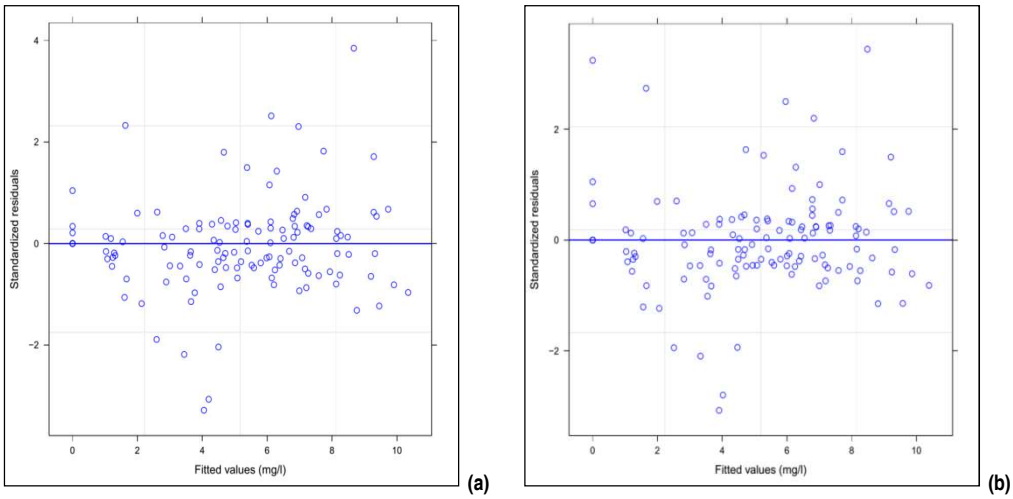


FIGURE 4: Plot of standardized residual errors against the fitted values of theophylline concentrations for model 3 **(a)** and model 4 **(b)**.

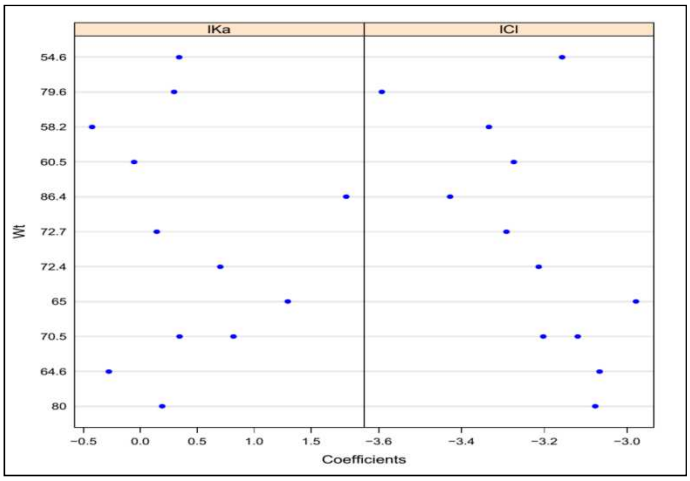


FIGURE 5: Plot of patients' weights against the estimated absorption rate and clearance (in logarithm scale) for all the 12 asthmatic patients.

The estimates of the PK parameters in the final chosen model (**model 4**) fitted to the Theophylline data have been reported in Table 2. Specifically, the estimates of the fixed effects components of clearance (C_l), absorption rate (k_a) and elimination rate (k_e) parameters (all in logarithm scales) as provided by the final model (**model 4**) are $\widehat{lk}_a$ = 0.4514, $\widehat{lk}_e$ = -2.4327 and $\widehat{lC}_l$ = -3.2145 respectively. Therefore, the estimated values of these PK parameters in their original scale as provided by the final model (**model 4**) are $\hat{k}_a$ =1.5420, $\hat{k}_e = 0.0860$ and $\hat{C}_l = 0.0397$ respectively.

The above estimates of the parameters ($\hat{k}_a$, $\hat{k}_e$, $\hat{C}_l$) summarized the pharmacokinetic processes for the population. The value of k_a indicates the fraction of theophylline present at the absorption site that is absorbed per hour. A value of k_a greater than 1 implies rapid absorption, that is, almost all the drug would be absorbed over the time interval. The estimated fixed effect component of k_a is 1.5420 hr^{-1} which indicates that, on the average, theophylline undergoes rapid absorption in the population of subjects. Also, k_e describes the change in the amount of theophylline in the body due to theophylline elimination over time. The estimated value of k_e of 0.0860 hr^{-1} implies that, on the average, 8.6% of theophylline remaining in the body is removed each hour. Furthermore, C_l describes the volume of

blood (or plasma) from which theophylline is eliminated per hour (drug loss from the body). A value of 0.0397 L/Kg/hr estimated for C_l implies that an average of 0.0397 Litres of blood is cleared of theophylline per hour.

Moreover, in addition to characterizing the average of the PK parameters in the population, the goal of PK modelling is to determine how the parameters (k_a, k_e, C_l) vary in the population of subjects in order to develop safe and effective drug regimens. If between-subject variation in (k_a, k_e, C_l) is large, it may be difficult to design a general routine treatment[14]. The estimated between-subject variations ink_a (0.6384) and C_l(0.1699) combined with experts' knowledge are useful for development of dosage strategies for administering theophylline to patients with respiratory diseases.

DISCUSSION AND CONCLUSION

The procedures for developing, estimating and analysing PK models using NLME techniques have been provided in this work. The NLME modelling technique is very suitable for modelling cluster dependent data such as those encountered in PK analysis. The subject-specific nature of NLME models makes them very suitable for handling pharmacokinetic models.

The modelling procedure adopted in this study started by plotting individual patient responses (theophylline concentration) against time as presented by Figure 1. This was done in order to have a clear overview of the pattern of the serum concentration over time. All the twelve line graphs in Figure 1 revealed the non-linear pattern of the theophylline serum concentration on all the asthmatic patients.

Results of separate non-linear regression models fitted on individual patient's theophylline data showed the behaviours of the three major PK parameters (absorption rate, elimination rate and clearance) in the models. The plots of the 95% confidence intervals of the estimates of the three PK parameters on individual patients as reported by Figure 2 suggested that the effects of elimination rate, k_e should be considered fixed while those of the absorption rate k_a and clearance, C_l should be considered mixed (having both fixed and random effects). All these notwithstanding, we considered the models' fitting to the data hierarchically until the best model that satisfies the needed required criteria is achieved.

A full NLME model where all the rates were considered mixed and the random effects variance-covariance matrix is positive definite was first fitted to the data, called model 1. Results from this model showed a high correlation between the random effects components of PK parameters C_l and k_e, which is an indication that the random effects variance-covariance matrix is ill-conditioned and that the requirement of independence between random effects parameters is not fulfilled. This led us to fit **model 2** that allowed the random effects variance-covariance matrix to be represented by a diagonal matrix in order to satisfy the requirement of independence.

The smaller BIC value of the simpler **model 2** compared to **model 1** confirms that **model 2** is a better model. However, the small standard deviation of the elimination rate k_e in the model further suggested that the effects of$k_e$in the model should be considered fixed. Therefore, a simpler **model 3** which defines k_e as a fixed effect was fitted and was found to be better as indicated by its smaller BIC value compared to **model 2**. However, the plot of the residual errors of **model 3** against the fitted values provided the evidence that homoscedasticity cannot be assumed for the residual errors in the model, therefore, **model 4** which facilitates modelling the errors with a combined additive (homoscedastic) and proportional error model was again fitted to the data.

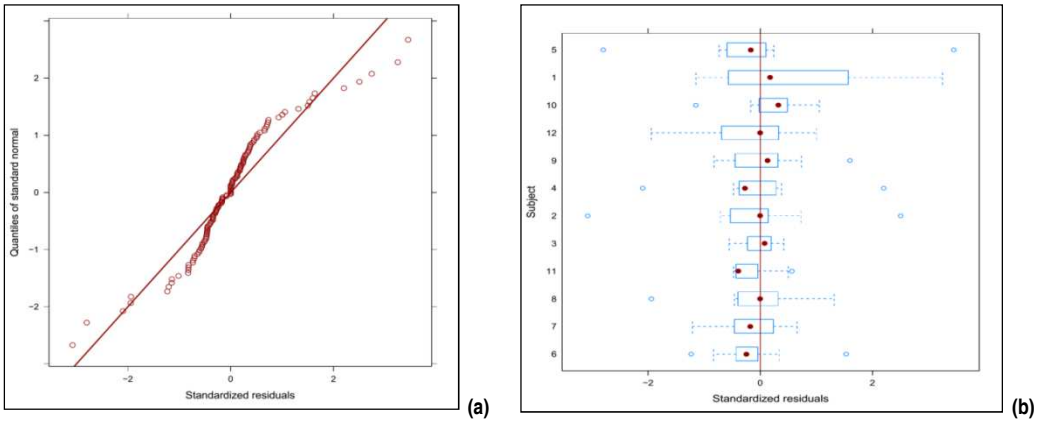
We investigated further if it is possible to improve the current **model 4** by including the weight variable as covariate in the model. This was done separately by fitting **models 5** and **6** in which the weight variable was incorporated into the model to replace the random effect components of clearance parameter (**model 5**) and absorption rate parameter (**model 6**). All the results obtained clearly indicated that model 4 with smallest BIC value was superior to both **models 5** and **6** with relatively higher BIC values compared to **model 4**. Hence, inclusion of weight as covariate in the PK model did not improve the efficiency of the model and was therefore ignored and the modelling process terminates.

Another point against the inclusion of weight in the model is that the oral dose (D) of theophylline administered on each of the asthmatic patients as incorporated in the final model (21) is weight adjusted. A real justification for this is clear in the high value of Pearson correlation coefficient between the oral dose of theophylline (D) and weight of the patients of -0.99 which is highly significant ($p < 0.0001$). Hence, the inclusion of weight as covariate in the model could trigger another problem of multicollinearity which obviously could render the model less efficient.

Without loss of generality, it can be concluded that the final NLME PK model that best fits the theophylline data discussed in this study is **model 4** which is governed by non-linear regression model (21) with the variance-covariance matrix of its error term ϵ_{ij} specified as in equations (4) and (5). This final model, as estimated, contains the fixed effects components of the clearance parameter (lC_l) β_1, absorption rate parameter (lk_a) β_2, and elimination rate parameter $(lk_e)\beta_3$.This modelalso contains the random effects component (b_{1i}) of the clearance parameter $lC_l$and random effects component (b_{3i}) of the absorption rate parameter lk_a. The above parameter mix in model (21) clearly indicated that only the effect of the absorption rate k_a was considered purely fixed in the model as earlier justified.

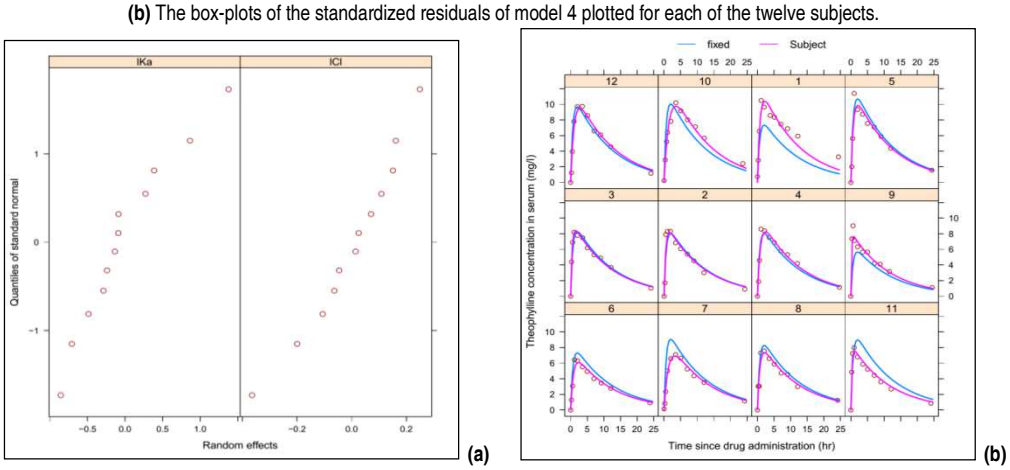
The estimates of the three PK parameters absorption rate (1.5420), elimination rate (0.0860) and clearance (0.0397) in the final model is an indication that these basic PK parameters are positive on the rate of theophylline concentration in asthmatic patients.

To further check for the adequacy of the final fitted model (**model 4**), we examined whether the error terms of the fitted model possesses the required normality assumption via its estimated residuals. The quantile-quantile (normal probability) plot and box-plots of the standardized residuals of the model as displayed in Figure 6a and b respectively showed that the normality assumption on the error term with mean zero is not severely violated. The box-plots of the standardised residuals of each subject were plotted as shown in Figure 6b to ensure clear overview of the behaviours of the residuals across the twelve subjects. The dotted points in the box-plots were the median residual values which were all quite close to the vertical line drawn on zero standardised residual in the plot. Although, the boxplots of the residuals as shown in Figure 6b generally suggest that there are outlying residual points in all the subjects or groups. However, this might be accounted for if the error terms in the models are assumed to follow a thick-tailed or skewed distribution such as the student-t, slash[37] or skew-normal[38,39] distributions instead of the normal distribution. This might be taken up in future study.



(a) (b)

FIGURE 6: (a) The quantile-quantile probability plot of the standardized residuals of the final model (model 4).
(b) The box-plots of the standardized residuals of model 4 plotted for each of the twelve subjects.



(a) (b)

FIGURE 7: (a) The quantile-quantile plots of the random effects of the absorption rate lk_a and clearance, lC_l.
(b) Plots of the fitted and actual theophylline concentrations for each of the twelve asthma patients.
The dot-lines are the actual (sample) values, the blue and pink lines represent the fixed/population and individual fitted values respectively.

It is also necessary to check if the estimated effects of the two random effects parameters$k_a$and $C_l$are normally distributed as required by the model. This was examined by plotting the quantile-quantile (Q-Q) plots of the estimated effects of the two parameters (in logarithmic scales) as shown in Figure 7a. It is obvious from the Q-Q plots in Figure 7a that the estimated effects of the two random effect parameters do not violate the normality assumption as required.

Finally, we examined the closeness of the estimated theophylline concentration with the observed ones in the samples based on the fitted model. This is achieved by plotting the fitted theophylline concentrations by the model superimposed on the actual concentrations (dotted lines) for all the twelve subjects as display in Figure 7b. The graphs in Figure 7b indicate that the individual fitted values are closer to the actual values than the fixed/population values, an indication that the NLME model fitted to the data is quite appropriate and efficient.

In conclusion, this study has provided a thorough review of techniques for fitting pharmacokinetic models within the framework of NLME methodology. The work has updated the basic procedures for analysing classical pharmacokinetic data for determining drug dosing strategies. However, the development and application of NLME models to pharmacokinetic data presented here is not exhaustive,

APPENDIX: The Theophylline published data 'Subject ID' is the number identifying the subject on whom the observation was made, 'Dose' is the dose of theophylline administered orally to the subject, 'Time' is the time since the drug was administered on the subject, 'Conc.' is the theophylline concentration in the subject and 'Wt' is the weight of the subject.[31]

Subject ID	Wt (Kg)	Dose (mg/Kg)	Time (hr)	Conc. (mg/L)	Subject ID	Wt (Kg)	Dose (mg)	Time (hr)	Conc. (mg/L)
1	79.6	4.02	0	0.74	7	64.6	4.95	0	0.15
1	79.6	4.02	0.25	2.84	7	64.6	4.95	0.25	0.85
1	79.6	4.02	0.57	6.57	7	64.6	4.95	0.5	2.35
1	79.6	4.02	1.12	10.5	7	64.6	4.95	1.02	5.02
1	79.6	4.02	2.02	9.66	7	64.6	4.95	2.02	6.58
1	79.6	4.02	3.82	8.58	7	64.6	4.95	3.48	7.09
1	79.6	4.02	5.1	8.36	7	64.6	4.95	5	6.66
1	79.6	4.02	7.03	7.47	7	64.6	4.95	6.98	5.25
1	79.6	4.02	9.05	6.89	7	64.6	4.95	9	4.39
1	79.6	4.02	12.12	5.94	7	64.6	4.95	12.05	3.53
1	79.6	4.02	24.37	3.28	7	64.6	4.95	24.22	1.15
2	72.4	4.4	0	0	8	70.5	4.53	0	0
2	72.4	4.4	0.27	1.72	8	70.5	4.53	0.25	3.05
2	72.4	4.4	0.52	7.91	8	70.5	4.53	0.52	3.05
2	72.4	4.4	1	8.31	8	70.5	4.53	0.98	7.31
2	72.4	4.4	1.92	8.33	8	70.5	4.53	2.02	7.56
2	72.4	4.4	3.5	6.85	8	70.5	4.53	3.53	6.59
2	72.4	4.4	5.02	6.08	8	70.5	4.53	5.05	5.88
2	72.4	4.4	7.03	5.4	8	70.5	4.53	7.15	4.73
2	72.4	4.4	9	4.55	8	70.5	4.53	9.07	4.57
2	72.4	4.4	12	3.01	8	70.5	4.53	12.1	3
2	72.4	4.4	24.3	0.9	8	70.5	4.53	24.12	1.25
3	70.5	4.53	0	0	9	86.4	3.1	0	0
3	70.5	4.53	0.27	4.4	9	86.4	3.1	0.3	7.37
3	70.5	4.53	0.58	6.9	9	86.4	3.1	0.63	9.03
3	70.5	4.53	1.02	8.2	9	86.4	3.1	1.05	7.14
3	70.5	4.53	2.02	7.8	9	86.4	3.1	2.02	6.33
3	70.5	4.53	3.62	7.5	9	86.4	3.1	3.53	5.66
3	70.5	4.53	5.08	6.2	9	86.4	3.1	5.02	5.67
3	70.5	4.53	7.07	5.3	9	86.4	3.1	7.17	4.24
3	70.5	4.53	9	4.9	9	86.4	3.1	8.8	4.11
3	70.5	4.53	12.15	3.7	9	86.4	3.1	11.6	3.16
3	70.5	4.53	24.17	1.05	9	86.4	3.1	24.43	1.12
4	72.7	4.4	0	0	10	58.2	5.5	0	0.24
4	72.7	4.4	0.35	1.89	10	58.2	5.5	0.37	2.89
4	72.7	4.4	0.6	4.6	10	58.2	5.5	0.77	5.22
4	72.7	4.4	1.07	8.6	10	58.2	5.5	1.02	6.41
4	72.7	4.4	2.13	8.38	10	58.2	5.5	2.05	7.83
4	72.7	4.4	3.5	7.54	10	58.2	5.5	3.55	10.21
4	72.7	4.4	5.02	6.88	10	58.2	5.5	5.05	9.18
4	72.7	4.4	7.02	5.78	10	58.2	5.5	7.08	8.02
4	72.7	4.4	9.02	5.33	10	58.2	5.5	9.38	7.14
4	72.7	4.4	11.98	4.19	10	58.2	5.5	12.1	5.68
4	72.7	4.4	24.65	1.15	10	58.2	5.5	23.7	2.42
5	54.6	5.86	0	0	11	65	4.92	0	0
5	54.6	5.86	0.3	2.02	11	65	4.92	0.25	4.86
5	54.6	5.86	0.52	5.63	11	65	4.92	0.5	7.24
5	54.6	5.86	1	11.4	11	65	4.92	0.98	8
5	54.6	5.86	2.02	9.33	11	65	4.92	1.98	6.81
5	54.6	5.86	3.5	8.74	11	65	4.92	3.6	5.87
5	54.6	5.86	5.02	7.56	11	65	4.92	5.02	5.22
5	54.6	5.86	7.02	7.09	11	65	4.92	7.03	4.45
5	54.6	5.86	9.1	5.9	11	65	4.92	9.03	3.62
5	54.6	5.86	12	4.37	11	65	4.92	12.12	2.69
5	54.6	5.86	24.35	1.57	11	65	4.92	24.08	0.86
6	80	4	0	0	12	60.5	5.3	0	0
6	80	4	0.27	1.29	12	60.5	5.3	0.25	1.25
6	80	4	0.58	3.08	12	60.5	5.3	0.5	3.96
6	80	4	1.15	6.44	12	60.5	5.3	1	7.82
6	80	4	2.03	6.32	12	60.5	5.3	2	9.72
6	80	4	3.57	5.53	12	60.5	5.3	3.52	9.75
6	80	4	5	4.94	12	60.5	5.3	5.07	8.57
6	80	4	7	4.02	12	60.5	5.3	7.07	6.59
6	80	4	9.22	3.46	12	60.5	5.3	9.03	6.11
6	80	4	12.1	2.78	12	60.5	5.3	12.05	4.57
6	80	4	23.85	0.92	12	60.5	5.3	24.15	1.17

as it is difficult for a single article to fully document the entire process and concept. Much attention has been focused on practical application of NLME modelling to a real life pharmacokinetic dataset, and we hope that this gives additional insight to readers already familiar with the NLME concept and provide a foundation for appreciating its principle and usefulness to those new to the concept. We have presented an analysis of a specific application using theophylline pharmacokinetics.

This work has only looked at the analysis of pharmacokinetic data using the NLME model from the frequentist approach. However, the development of NLME model for analysing pharmacokinetic data can be approached from Bayesian perspective through the incorporation of necessary prior information in the model.[40,41] In future studies, we hope to continue in developing methodologies for NLME models within both the Frequentist and Bayesian paradigm for better efficiency.

Acknowledgement

The authors thank the two anonymous reviewers for their valuable comments on the first draft of this paper.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Isaac Adeola Adeniyi, Waheed Babatunde Yahya;* ***Design:*** *Isaac Adeola Adeniyi, Waheed Babatunde Yahya;* ***Control/Supervision:*** *Waheed Babatunde Yahya;* ***Data Collection and/or Processing:*** *Isaac Adeola Adeniyi, Waheed Babatunde Yahya, Chinenye Pauline Ezenweke;* ***Analysis and/or Interpretation:*** *Isaac Adeola Adeniyi, Waheed Babatunde Yahya, Chinenye Pauline Ezenweke;* ***Literature Review:*** *Isaac Adeola Adeniyi, Waheed Babatunde Yahya;* ***Writing The Article:*** *Isaac Adeola Adeniyi, Waheed Babatunde Yahya;* ***Critical Review:*** *Waheed Babatunde Yahya;* ***References and Fundings:*** *Waheed Babatunde Yahya.*

REFERENCES

1. Sheiner LB, Ludden TM. Population pharmacokinetics/dynamics. Annu Rev Pharmacol Toxicol 1992;32:185-209.
2. Karger AG. SOP 13: pharmacokinetic data analysis. Oncologie 2003;26 Suppl 6:56-9.
3. Wakefield J, Aarons L, Racine-Poon A. The Bayesian approach to population pharmacokinetic/pharmacodynamic modelling. Case Studies in Bayesian Statistics 1999;140:205-65.
4. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. II. Biexponential model and experimental pharmacokinetic data. J Pharmacokinet Biopharm 1981;9(5):635-51.
5. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. J Pharmacokinet Biopharm 1980;8(6):553-71.
6. Lindstrom ML, Bates DM. Nonlinear mixed-effects models for repeated measures data. Biometrics 1990;46(3):673-87.
7. Pinheiro J. Topics in Mixed Effects Models. Ph.D. thesis. University of Wisconsin-Madison, WI; 1994. p.210.
8. Wu L. Mixed Effects Models for Complex Data. 1st ed. Taylor & Francis, FL: Chapman & Hall/CRC; 2010. p.431.
9. Pinheiro J, Bates D. Mixed-Effects Models in S and S-PLUS. 1st ed. New York: Springer; 2000. p.528.
10. Tierney L, Kadane JB. Accurate approximations for posterior moments and densities. J Am Stat Assoc 1986;81(393):82-6.
11. Geweke J. Bayesian inference in econometric models using Monte Carlo integration. Econometrica 1989;57(6):1317-39.
12. Davidian M, Gallant AR. Smooth nonparametric maximum likelihood estimation for population pharmacokinetics, with application to guanidine. J Pharmacokinet Biopharm 1992;20:529-56.
13. Zhou M. Fully exponential laplace approximation EM algorithm for nonlinear mixed effects models. Ph.D. Dissertation. University of Nebraska; 2009. p.204.
14. Davidian M, Giltinan DM. Nonlinear models for repeated measurement data: an overview and update. J Agric Biol Environ Stat 2003;8:387-419.
15. Sakamoto Y, Ishiguro M, Kitagawa G. Akaike Information Criterion Statistics. 1st ed. Tokyo: D. Reidel Publishing Company; 1986. p.290.
16. Schwarz G. Estimating the dimension of a model. Ann Stat 1978;6(2):461-4.
17. Lehmann EL. Testing Statistical Hypotheses. 2nd ed. New York: Wiley; 1986. p.600.
18. Pinheiro J, Bates DM. Approximations to the log-likelihood function in the nonlinear mixed effects model. J Comput Graph Stat 1995;4(1):12-35.

19. Davidian M, Giltinan DM. Nonlinear Models for Repeated Measurement Data. 1st ed. Boca Raton, Florida: Chapman & Hall/CRC Press; 1995. p.360.
20. Beal SL, Sheiner LB. NONMEM User's Guides. 1st ed. University of California, San Francisco: NONMEM Project Group; 1994.
21. Pinheiro J, Bates D, DebRoy S, Sarkar D. The R core team 'NLME': linear and nonlinear mixed effects models. R Package Version 2017;3:1-131.
22. Gelman A, Bois F, Jiang J. Physiological pharmacokinetic analysis using population modelling and informative prior distributions. J Am Stat Assoc 1996;91(436):1400-12.
23. Gibaldi M, Perrier D. Pharmacokinetics. Revised and Expanded: Drugs and the Pharmaceutical Sciences. 2nd ed. Informal Healthcare; 2007. p.15.
24. Derendorf H, Lesko JL, Chaikin P, Colburn WA, Lee P, Miller R, et al. Pharmacokinetic/pharmacodynamic modelling in drug research and development. J Clin Pharmacol 2000;40(12 Pt 2):1399-418.
25. DiPiro J, Spruill W, Wade W, Blouin R. Concepts in Clinical Pharmacokinetics. 5th ed. Bethesda: American Society of Health-System Pharmacists; 2010. p.248.
26. Kramer WG, Lewis RP, Cobb TC, Forester WF Jr, Visconti JA, Wanke LA, et al. Pharmacokinetics of digoxin: comparison of a two- and a three-compartment model in man. J Pharmacokinet Biopharm 1974;2(4):299-312.
27. David S, Zaizai L. Population pharmacokinetics studies with nonlinear mixed effects modelling. SAS Global Forum 2007;148.
28. Younggil K. Handbook of Essential Pharmacokinetics, Pharmacodynamics and Drug Metabolism for Industrial Scientists. 1st ed. New York: Kluwer Academic Publishers; 2002. p.291.
29. Barnes PJ, Pauwels RA. Theophylline in the management of asthma: time for reappraisal? Eur Respir J 1994;7(3):579-91.
30. Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker; 1982. p.504.
31. Boeckmann AJ, Sheiner LB, Beal SL. NONMEM Users Guide: Part V. 1st ed. University of California, San Francisco: NONMEM Project Group; 1994.
32. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015. URL http://www.R-project.org/.
33. Geweke JF, Meese RA. Estimating regression models of finite but unknown order. International Economics Review 1981;22:55-70.
34. Katz RW. On some criteria for estimating the order of a Markov chain. Technometrics 1981;23(3):243-9.
35. Koehler AB, Murphree ES. A comparison of the Akaike and Schwarz criteria for selecting model order. Applied Statistics 1988;37(2):187-95.
36. Yahya WB, Kolade EI, Garba MK, Usman A. Power analysis of the likelihood ratio tests for exponential populations. The Transactions of Mathematical Physics 2017;3:123-42.
37. Rosa GJM, Padovani CR, Gianola D. Robust linear mixed models with normal/independent distributions and Bayesian MCMC implementation. Biom J 2003;45:573-90.
38. Azzalini A, Dalla-Valle A. The multivariate skew-normal distribution. Biometrika 1996;83(4):715-26.
39. Yahya WB, Rosenberg R, Ulm K. Microarray-based classification of histopathologic responses of locally advanced rectal carcinomas to neoadjuvant radiochemotherapy treatment. Turkiye Klinikleri J Biostat 2014;6(1):8-23.
40. Wakefield J, Smith A, Racine-Poon A, Gelfand A. Bayesian analysis of linear and nonlinear population models using the Gibbs sampler. Applied Statistics 1994;43:201-21.
41. Gelman A, Carlin JB, Stern HS, Rubin DB. Bayesian Data Analysis. 2nd ed. Boca Raton: Chapman and Hall/CRC Press; 2004. p.690.