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ACKNOWLEDGMENTS

Abstracted in part from a dissertation submitted by P. M. Kabra to the University of Kansas in partial fulfillment of the Doctor of Philosophy degree requirements.

Kinetic Parameter Estimation by Numerical Algorithms and Multiple Linear Regression: Application to Pharmacokinetics

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Abstract □ Two numerical examples are presented to illustrate the application of the proposed method of parameter estimation in pharmacokinetics. Results for a system exemplifying first-order kinetics indicate that parameters estimated by the proposed procedure compare favorably with those estimated by a nonlinear regression method. In a simulated example characterized by Michaelis–Menten elimination kinetics, the accuracy of the estimated parameters was comparable to that expected, verifying the validity of the method. The importance of the numerical approximation algorithms was demonstrated also.

Keyphrases □ Pharmacokinetics—estimation by numerical algorithms and multiple linear regression □ Numerical algorithms—estimation of kinetic parameters □ Multiple linear regression—estimation of kinetic parameters □ Statistics—numerical algorithms, multiple linear regression, estimation of kinetic parameters

A general approach suitable for parameter estimation was reported previously (1). The strategy is to obtain equivalent mathematical expressions in linear form. Briefly, the procedure involves data transformation, usually by numerical integration and/or differentiation, followed by multiple linear regression. The application of this technique in pharmacokinetics is illustrated in the present report.

ESTIMATION PROCEDURE

Linear Case—Many pharmacokinetic processes are linear, and their description can be approximated by multiexponential equations. In a two-compartment open model (2, 3), the observed drug concentration in the central compartment, C_i, after intravenous administration can be described by:

$$C_i = a\exp(-\alpha t_i) + b\exp(-\beta t_i) \qquad i = 1, 2, \ldots, n \qquad \text{(Eq. 1)}$$

where a, b, α, and β are unknown constants and n is the number of observations[1]. If the elimination is assumed to take place from the central compartment only, as depicted in Scheme I, the relationships between the four constants and the model parameters become:

$$\alpha = 0.5[(k_{12} + k_{21} + k_{10}) + \sqrt{(k_{12} + k_{21} + k_{10}) - 4k_{21}k_{10}}] \qquad \text{(Eq. 2)}$$

$$\beta = 0.5[(k_{12} + k_{21} + k_{10}) - \sqrt{(k_{12} + k_{21} + k_{10}) - 4k_{21}k_{10}}] \qquad \text{(Eq. 3)}$$

$$a = D(\alpha - k_{21})/V(\alpha - \beta) \qquad \text{(Eq. 4)}$$

[1] For simplicity, subscript i is omitted and implied in most of the equations.

$$T \underset{K_{21}}{\overset{K_{12}}{\rightleftharpoons}} B \xrightarrow{K_{10}} U$$

Scheme I—Linear two-compartment open model depicting the body as composed of the central compartment B *(including blood) and the peripheral compartment* T, *where* k_{12} *and* k_{21} *are first-order intercompartmental transfer rate constants,* k_{10} *is the first-order elimination rate constant, and* U *is the eliminating compartment. The drug dose* D *is introduced into the central compartment at zero time. The drug concentration in the central compartment is defined as* C = B/V, *where* V *is the volume of distribution of the central compartment.*

$$b = D(k_{21} - \beta)/V(\alpha - \beta) \qquad \text{(Eq. 5)}$$

$$V = D/(a + b) \qquad \text{(Eq. 6)}$$

By applying the procedure described earlier (1) and recognizing that concentration at time zero is not an observation for a given experiment, the biexponential equation can be transformed into the following linear expression, which contains four terms:

$$C = \sum_{j=1}^{4} A_j X_j \qquad \text{(Eq. 7)}$$

where:

$$A_1 = \frac{D}{V} + \frac{K_{21}D}{V}t_1 - (\alpha + \beta)\int_0^{t_1} C\,dt - (\alpha\beta)\int_0^{t_1}\int_0^{t_1} C\,dt^2 \qquad \text{(Eq. 8)}$$

$$A_2 = \frac{K_{21}D}{V} - (\alpha\beta)\int_0^{t_1} C\,dt \qquad \text{(Eq. 9)}$$

$$A_3 = -(\alpha + \beta) \qquad \text{(Eq. 10)}$$

$$A_4 = -(\alpha\beta) \qquad \text{(Eq. 11)}$$

$$X_1 = 1.0 \qquad \text{(Eq. 12)}$$

$$X_2 = t_i - t_1 \qquad \text{(Eq. 13)}$$

$$X_3 = \int_{t_1}^{t_i} C\,dt \qquad \text{(Eq. 14)}$$

$$X_4 = \int_{t_1}^{t_i} X_3\,dt \qquad \text{(Eq. 15)}$$

In deriving Eqs. 8 and 9, the following identities apply:

$$\int_0^{t_i} C\,dt = \int_0^{t_1} C\,dt + \int_{t_1}^{t_i} C\,dt \qquad \text{(Eq. 16)}$$

$$\int_0^{t_i}\int_0^{t_i} C\,dt^2 = \int_0^{t_1}\int_0^{t_1} C\,dt^2 + (t_i - t_1)\cdot\int_0^{t_1} C\,dt + \int_{t_1}^{t_i}\int_{t_1}^{t_i} C\,dt^2 \qquad \text{(Eq. 17)}$$

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$$T \underset{K_{21}}{\overset{K_{12}}{\rightleftharpoons}} B \xrightarrow{V_m, K_m} U$$

Scheme II—Nonlinear disposition model obtained by modification of Scheme I. Conditions are identical for the two schemes except that the elimination is characterized by saturable Michaelis–Menten-type kinetics.

Equation 7 also can be independently derived from the following rate and mass-balance equations:

$$\dot{C} = -(k_{12} + k_{10})C + (k_{21}/V)T \quad \text{(Eq. 18a)}$$

$$\dot{U} = k_{10}VC \quad \text{(Eq. 18b)}$$

$$D = VC + T + U \quad \text{(Eq. 18c)}$$

From Eqs. 8–11, where A_1 and A_2 are simplified to:

$$A_1 = a \exp(-\alpha t_1) + b \exp(-\beta t_1) \quad \text{(Eq. 19)}$$

$$A_2 = a\beta \exp(-\alpha t_1) + b\alpha \exp(-\beta t_1) \quad \text{(Eq. 20)}$$

the four parameters can be calculated:

$$\alpha = 0.5[-A_3 + (A_3^2 + 4A_4)^{1/2}] \quad \text{(Eq. 21)}$$

$$\beta = 0.5[-A_3 - (A_3^2 + 4A_4)^{1/2}] \quad \text{(Eq. 22)}$$

$$K_{21} = \frac{\beta(A_2 - A_1\alpha)\exp(-\beta t_1) - \alpha(A_2 - A_1\beta)\exp(-\alpha t_1)}{(A_2 - A_1\alpha)\exp(-\beta t_1) - (A_2 - A_1\beta)\exp(-\alpha t_1)} \quad \text{(Eq. 23)}$$

$$V = \frac{D}{A_1(\alpha - \beta)}[(\alpha - k_{21})\exp(-\alpha t_1) + (k_{21} - \beta)\exp(-\beta t_1)] \quad \text{(Eq. 24)}$$

While four parameters are associated with the biexponential equation, only α and β are nonlinearly related. The other two constants, a and b, are linearly related and can be estimated by linear regression. Thus, the procedure is slightly modified so that regression Eq. 7 is used to obtain the two nonlinear parameters only. Once they are calculated from Eqs. 21 and 22, they are then substituted into Eq. 1 to obtain a and b by a second linear regression.

Nonlinear Case—A nonlinear pharmacokinetic model obtained by a simple modification of the previous one is shown in Scheme II. In this model, drug elimination from the central compartment is assumed to follow Michaelis–Menten kinetics. The rate and mass-balance equations are:

$$\dot{C} = -k_{12}C + (k_{21}/V)T - (V_m/V)C/(K_m + C) \quad \text{(Eq. 25a)}$$

$$\dot{U} = V_mC/(K_m + C) \quad \text{(Eq. 25b)}$$

$$D = VC + T + U \quad \text{(Eq. 25c)}$$

where V_m is the maximum rate and K_m is the Michaelis constant.

Although closed-form solutions explicit in t to this nonlinear model cannot be obtained, numerical solutions can be generated by using Runge–Kutta approximations.

The regression equation describing the time course of the drug concentration in the central compartment is:

$$C = \sum_{j=1}^{4} A_jX_j \quad \text{(Eq. 26)}$$

where:

$$A_1 = \frac{k_{21}D}{V(k_{12} + k_{21})} - \frac{k_{21}V_m}{V(k_{12} + k_{21})}\int_0^{t_1} \frac{C}{K_m + C}\,dt \quad \text{(Eq. 27)}$$

$$A_2 = -1/(k_{12} + k_{21}) \quad \text{(Eq. 28)}$$

$$A_3 = -V_m/V(k_{12} + k_{21}) \quad \text{(Eq. 29)}$$

$$A_4 = -k_{21}V_m/V(k_{12} + k_{21}) \quad \text{(Eq. 30)}$$

$$X_1 = 1.0 \quad \text{(Eq. 31)}$$

$$X_2 = \dot{C} \quad \text{(Eq. 32)}$$

$$X_3 = C/(K_m + C) \quad \text{(Eq. 33)}$$

$$X_4 = \int_{t_1}^{t_i} X_3\,dt \quad \text{(Eq. 34)}$$

The main difference between the two models is the presence of nonlinear parameter K_m. As described earlier (1), the best-fit K_m can be found by scanning the weighted sum of squared deviations, WSS, as a function of K_m. The minimum WSS is then located by iteration. The search should be confined to a range of values where K_m is numerically comparable to the observed C values. Otherwise, the system reduces to a linear model with either first-order ($K_m \gg C$) or zero-order ($K_m \ll C$) elimination.

Numerical Integration—To perform the regression analysis, numerical methods such as numerical integration and numerical differentiation are required to generate the necessary X_j values. Many numerical integrating algorithms, including the well-known trapezoidal rule, can be found in the literature (5). In a recent publication, algorithms based on Lagrange and spline functions were shown to be relatively free of systematic errors (6).

A slightly modified spline method is employed in the present work to minimize spurious oscillations near both ends of the curve. In the original procedure (6), the two end conditions are defined as $\ddot{Y}_2 = \ddot{Y}_3$ and $\ddot{Y}_{n-1} = \ddot{Y}_n$. In the modified version, the first condition is amended so that $\ddot{Y}_1 = 0$ if $Y_1 = 0$, with the constraint $\dot{Y}_1 \geq 0$. The second condition is changed to $\ddot{Y}_n = 0$; in the event that $Y_n = 0$, an additional constraint of $\dot{Y}_n = 0$ is also imposed. These two conditions, except for the constraints, were suggested previously (7). The modified procedure is especially useful if input data contain two or more consecutive zero Y values.

Numerical Differentiation—Numerical differentiation is required to generate the X_2 values in Eq. 32. In contrast to numerical integration, which tends to dampen noise, numerical differentiation tends to magnify the effect of input errors. If experimental data are of high precision, interpolating polynomials such as spline functions (6, 7) can be used. With noisy data, a possible solution is to use smoothing least-squares polynomials. The procedure is described here.

To obtain derivatives at each data point (t_i, C_i), the nearest five contiguous points are taken and fitted with a cubic polynomial, using an appropriate weighting scheme:

$$C = a_i + b_it + c_it^2 + d_it^3 \quad \text{(Eq. 35)}$$

The four coefficients are solved by the standard linear regression technique. Once they are obtained, the derivative of Eq. 35 is evaluated at t_i:

$$\dot{C}_i = b_i + 2c_it_i + 3d_it_i^2 \quad \text{(Eq. 36)}$$

This procedure is repeated successively for each i, $i = 3, 4, \ldots, n-2$. For $i = 1$ or 2, the derivative is calculated from the cubic polynomial fitted to the first five points. For $i = n-1$, it is calculated from the cubic polynomial fitted to the last five points. For $i = n$, it is calculated from the following equation, assuming monoexponential decay of C between t_{n-1} and t_n:

$$\dot{C}_n = C_n \ln (C_n/C_{n-1})/(t_n - t_{n-1}) \quad \text{(Eq. 37)}$$

Derivatives obtained in this procedure are less sensitive to the variation in the data than those obtained by the spline method. However, since a cubic polynomial allows one inflection point and two extrema, approximations are usually less accurate if experimental errors are high.

To improve the reliability, a second set of derivatives is calculated by repeating the entire procedure, using parabolic polynomials as the fitting function. The corresponding fitting polynomials and derivatives are:

$$C = a_i + b_it + c_it^2 \quad \text{(Eq. 38)}$$

$$\dot{C}_i = b_i + 2c_it_i \quad \text{(Eq. 39)}$$

The X_2 values as shown in Eq. 32 are obtained by taking the average of the two derivatives calculated from the above two series. This procedure is called the LEASQ method.

Weighting Scheme—Since there are diversified analytical techniques for obtaining pharmacokinetic data, experimental observations may not be homogeneous in variance at all times. These variances should be considered when devising a weighting scheme. In general, data are weighted in proportion to the inverse of their variances. Excellent discussions of data weighting can be found elsewhere (8).

Accuracy of Parameters—By analogy to a technique commonly used in nonlinear regression, the uncertainty associated with each estimated parameter can be evaluated as follows. If $\overline{D}$ is a square matrix of the weighted sum of cross-products of the approximate partial derivatives, then the approximate standard error, ASE, of parameter θ_j is:

$$ASE(\theta_j) = \sqrt{d_{jj}WSS/df} \quad \text{(Eq. 40)}$$

Table I—Comparison of the Present Results and Those Obtained by the Nonlinear Regression Program COMPT [a]

Parameter	Ref. 10	Present Results[b]		
		Spline	Lagrange	Trapezoidal
k_{12}, hr^{-1}	0.160	0.136	0.145	0.172
k_{21}, hr^{-1}	0.345	0.247	0.245	0.454
k_{10}, hr^{-1}	0.570	0.545	0.551	0.579
α, hr^{-1}	0.844	0.747 (0.115)	0.764 (0.111)	0.920 (0.220)
β, hr^{-1}	0.235	0.180 (0.020)	0.177 (0.019)	0.286 (0.013)
V, liters	7.59	7.83	7.74	7.62
V_p, liters/hr	4.35	4.27	4.27	4.41
a, μg/ml	53.94	56.32 (16.91)	57.08 (15.00)	48.23 (21.12)
b, μg/ml	11.96	7.52 (14.24)	7.51 (12.65)	17.41 (17.85)
A_1, μg/ml	—	56.31	56.61	57.05
A_2, μg/ml/hr	—	14.34	14.32	28.23
A_3, hr^{-1}	—	−0.927	−0.940	−1.206
A_4, hr^{-2}	—	−0.134	−0.135	−0.263
WSS	0.0245	0.0230	0.0242	0.0283

[a] Dose = 500 mg. [b] *ASE* values are given in parentheses.

Table II—Tabulation of Time-Dependent Variables, where X_3 and X_4 Values Were Generated by the Spline Method, for the Spectinomycin Data

i	t, hr	C, μg/ml	X_1	X_2, hr	X_3, μg hr/ml	X_4, μg hr^2/ml
1	0.1667	63.3	1.0	0	0	0
2	0.3333	50.6	1.0	0.1666	9.3864	0.7972
3	0.5	43.3	1.0	0.3333	17.1643	3.0314
4	1.0	31.0	1.0	0.8333	35.3746	16.4102
5	2.0	18.3	1.0	1.8333	59.3086	64.8208
6	4.0	6.9	1.0	3.8333	82.5641	210.5057
7	6.0	3.05	1.0	5.8333	91.6509	385.9920
8	8.0	1.95	1.0	7.8333	96.5691	574.4830

Table III—Summary of the Estimated Parameters for the Nonlinear Model, Using Derivatives Obtained by the Spline Method or by the LEASQ Method [a]

Parameter	True Value	Error-Free Data[b]		Corrupted Data[b]		
		Spline	LEASQ	Spline	LEASQ	Expected
K_m, μg/ml	4.0	4.095 (0.039)	2.192 (0.532)	3.786 (4.430)	2.277 (2.204)	4.0 (4.293)
V_m, mg/hr	40.0	40.592 (0.234)	29.491 (3.672)	38.499 (26.849)	28.774 (14.652)	40.0 (25.742)
V, liters	10.0	10.003 (0.004)	10.128 (0.118)	8.658 (0.558)	9.459 (0.448)	10.0 (0.453)
k_{12}, hr^{-1}	1.0	0.998 (0.001)	0.966 (0.023)	1.432 (0.214)	1.086 (0.104)	1.0 (0.105)
k_{21}, hr^{-1}	0.5	0.502 (0.001)	0.422 (0.027)	0.557 (0.155)	0.409 (0.096)	0.5 (0.125)
WSS	—	1.73×10^{-6}	1.56×10^{-3}	5.57×10^{-2}	3.01×10^{-2}	2.41×10^{-2}

[a] Dose = 200 mg. [b] *ASE* values are given in parentheses.

where d_{jj} is the jth diagonal element of the inverse matrix $\overline{D}^{-1}$ and df is the degree of freedom. By definition, the elements of the matrix $\overline{D}$ are:

$$a_{jl} = \sum_{i=1}^{n} W_i \cdot \frac{\partial C_i}{\partial \theta_j} \cdot \frac{\partial C_i}{\partial \theta_l} \qquad j,l = 1, 2, \ldots, p \qquad \text{(Eq. 41)}$$

where W_i is the weight of C_i, and p is the number of parameters. If Δ is small compared to θ_j, the partial derivatives can be approximated by:

Table IV—Comparison of X_2 Values (Micrograms per Milliliter per Hour) Calculated by the Spline or the LEASQ Method from Simulated Data Containing 10% Random Noise

t, hr	Theoretical	Spline	LEASQ
0.1	−20.1748	−30.6219	−22.7207
0.2	−17.4505	−24.6711	−19.2046
0.5	−11.3241	−10.1656	−10.8613
1.0	−5.5902	−4.3048	−5.9614
1.5	−2.8722	−4.1152	−3.2711
2.0	−1.6013	−0.5344	−1.9930
3.0	−0.7330	−1.4030	−1.1104
4.0	−0.5188	−0.5738	−0.6446
6.0	−0.3718	−0.2766	−0.3547
8.0	−0.2695	−0.2699	−0.2428
10.0	−0.1875	−0.1438	−0.1715
12.0	−0.1265	−0.1449	−0.1334
14.0	−0.0838	−0.0717	−0.0887
16.0	−0.0549	−0.0496	−0.0496

$$\frac{\partial C_i}{\partial \theta_j} = \frac{C_i(\theta_1, \ldots, \theta_j + \Delta, \ldots, \theta_p) - C_i(\theta_1, \ldots, \theta_j - \Delta, \ldots, \theta_p)}{2\Delta} \qquad \text{(Eq. 42)}$$

Equation 42, when applied to multiexponential functions of the form:

$$C_i = \sum_{k=1}^{m} a_k \exp(-\lambda_k t_i) \qquad \text{(Eq. 43)}$$

can be simplified to yield:

$$\frac{\partial C_i}{\partial a_k} = \exp(-\lambda_k t_i) \qquad \text{(Eq. 44)}$$

$$\frac{\partial C_i}{\partial \lambda_k} = \frac{-a_k t_i}{2\Delta}[e^{-(\lambda_k+\Delta)t_i} - e^{-(\lambda_k-\Delta)t_i}] \qquad \text{(Eq. 45)}$$

EXPERIMENTAL

Computation—A computer program, written in FORTRAN, was devoloped to make the necessary computations[2]. Briefly, the program contains several general subroutines to perform the following tasks: receive input, compute weights, numerically integrate and differentiate by the described procedures, compile matrix elements, compute determinant, obtain inverse matrix, calculate regression coefficients, solve a system of simultaneous linear equations, locate the minimum by qua-

[2] Detailed description of the program will be documented elsewhere.

Table V—Effect of K_m on *WSS* and Other Model Parameters Using Corrupted Data Set and X_2 Values Calculated by the LEASQ Method

K_m, μg/ml	K_{12}, hr^{-1}	K_{21}, hr^{-1}	V_m, mg/hr	V, liters	*WSS*
1.0	1.221	0.248	31.72	4.982	2.046
1.5	1.172	0.310	30.83	6.476	0.597
2.0	1.119	0.371	29.70	8.233	0.094
2.5	1.056	0.442	27.83	10.663	0.070
3.0	0.969	0.537	25.03	14.553	0.474
3.5	0.834	0.681	21.24	21.836	1.443
4.0	0.583	0.945	16.44	38.533	3.332
4.5	−0.062	1.604	—	—	—

dratic approximation, and compute *ASE* values associated with multiexponential equations. In addition, specific subroutines were developed to generate numerical solutions to Eqs. 25*a*–25*c* and to compute the associated *ASE* values. The value of Δ was arbitrarily taken to be $0.001\theta_j$.

Linear Case—A set of spectinomycin serum data, originally published by Wagner *et al.* (9), was used to demonstrate the estimation procedure and to compare the results with those reported in the literature. The same data were cited by Pfeffer (10) to illustrate the nonlinear regression program COMPT, assuming the model depicted in Scheme I.

To make the results comparable, the data were weighted by $1/C^2$ as in COMPT. The two-step regression procedure was repeated three times to test the effect of the numerical integrating algorithms (6) on the estimated parameter values.

In the first run, X_3 values were calculated by the log trapezoidal method and X_4 values were calculated by the linear trapezoidal method. In the second run, both were calculated by the Lagrange method. In the third run, both were calculated by the modified spline method.

Nonlinear Case—Two simulated data sets, one error free and the other corrupted with 10% random noise, were generated by the fourth-order Runge–Kutta technique to illustrate the iterative nonlinear estimation procedure. Two numerical differentiation algorithms, the spline method and the LEASQ method with weights defined in Eq. 46, were employed to generate the X_2 values so that the effect of data smoothing on parameter estimation could be examined. In all cases, the spline method was employed to generate X_4 values and data were weighted according to Eq. 46, where the sum of weights was equal to the number of data points (11):

$$W_i = (n/C_i^2) \Big/ \sum_{i=1}^{n} (1/C_i^2) \qquad \text{(Eq. 46)}$$

The *WSS* was first scanned as a function of K_m in the range of 1–10 μg/ml, with the constraint that all model parameters must be positive. The more precise location of the best-fit K_m was then refined by iteration. Convergence was assumed if, in two consecutive iterations, the change in K_m was less than 0.01% by quadratic approximation. At each iteration, the four linear parameters were calculated from the regression coefficients as follows:

$$k_{21} = -A_4/A_3 \qquad \text{(Eq. 47)}$$

$$k_{12} = -k_{21} - 1/A_2 \qquad \text{(Eq. 48)}$$

$$V = A_2 D \Big/ \left(A_3 \int_0^{t_1} \frac{C}{K_m + C}\, dt - A_1/k_{21} \right) \qquad \text{(Eq. 49)}$$

$$V_m = V(A_3/A_2) \qquad \text{(Eq. 50)}$$

where V was further adjusted to yield the lowest *WSS* value.

RESULTS AND DISCUSSION

Linear Case—Table I lists the results, including the four intermediate regression coefficients defined in Eqs. 8–11, of pharmacokinetic analysis for the spectinomycin data. The effect of numerical algorithm is obvious. While the estimated parameters and the corresponding *ASE* values are generally comparable by all three methods, a comparison of the *WSS* values suggests that the spline method gave the best fit, followed by the Lagrange method, and then by the trapezoidal method. These differences are related to the algorithm errors associated with the numerical integration procedures used. Listed in Table II are the X_3 and X_4 values and other time-dependent variables. Plotted in Fig. 1 are the interpolating spline functions for C and X_3. Evidently, no spurious oscillations occurred with this sample (6).

For comparison, results of nonlinear regression analysis on the same data used by Pfeffer (10) are listed in the second column of Table I. With the exception of V, parameter values by COMPT were intermediate between those of the trapezoidal method and those of the Lagrange method. On the basis of *WSS* values, the present method is either comparable to, or slightly better than, the nonlinear regression process, depending on the numerical integrating algorithm employed. The two-step regression procedure improved the fitting by reducing the effect of algorithm errors, as evidenced by comparing the present results with those reported earlier (12), and is applicable to other multiexponential equations. In some cases, either the trapezoidal or the Lagrange procedure may yield lower *WSS* values.

Nonlinear Case—Shown in Table III are the parameter values used in generating simulated data and the results of analysis in accordance with the nonlinear model. When employing collocative splines as the numerical differentiation tool, it is apparent that nearly perfect solutions were obtained with error-free data as the input. In contrast, solutions based on the LEASQ numerical differentiation algorithms were relatively poor, suggesting imprudence in trying to improve upon perfection.

Results for data corrupted with random noise are shown in columns 4 and 5 of Table III. On the basis of *WSS* values, the LEASQ algorithm appears to have yielded better solutions. This result is related to the basic differences between the two approximating procedures, the LEASQ method being more accurate in generating derivatives from data containing noise. Comparisons of the calculated X_2 values are given in Table IV.

The effect of K_m on *WSS* is exemplified in Table V. Corresponding values for other model parameters are also listed. As can be seen, increasing K_m caused the formation of a minimum in *WSS*. A further increase in K_m resulted in the formation of meaningless solutions.

With noisy data (Table III), neither algorithm produced solutions that were consistently closer to the expected true values. The uncertainty of the estimated parameters can be examined by comparing the *ASE* values with those predicted from the expected parameter values (13). The proximity of the actual to the predicted errors is a measure of the reli-

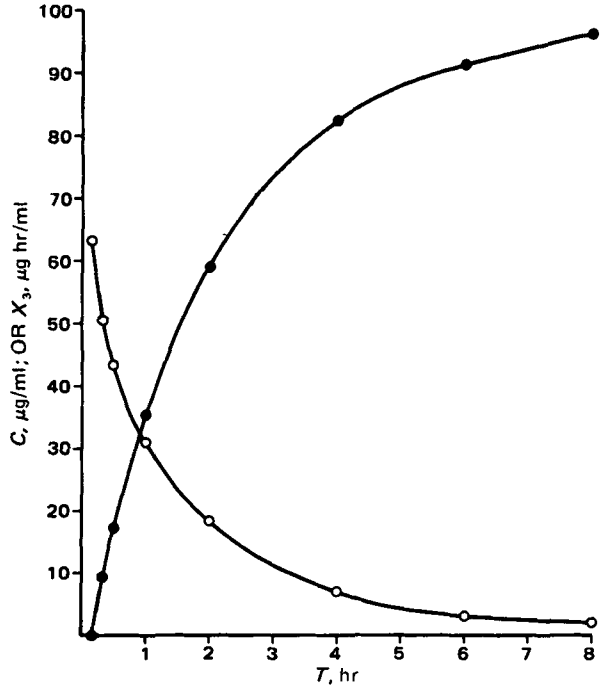

Figure 1—*Spline function interpolation of* C (○) *and* X_3 (●) *for spectinomycin data.*

Table VI—Comparison of the Predicted and Input Data

t, hr	Input[a]	Predicted by Spline[b]	Predicted by LEASQ[b]	Theoretical (Expected)[b]
0.1	18.68	19.7470 (−4.26)	18.7512 (−0.28)	17.8285 (3.40)
0.2	15.92	16.9891 (−5.01)	16.6838 (−3.58)	15.9506 (−0.14)
0.5	10.82	11.3095 (−3.37)	12.0142 (−8.23)	11.7020 (−6.08)
1.0	8.38	6.8147 (13.92)	7.5799 (7.12)	7.6489 (6.50)
1.5	5.57	5.0242 (7.30)	5.3858 (2.47)	5.6179 (−0.64)
2.0	4.83	4.2152 (9.49)	4.2544 (8.89)	4.5397 (4.48)
3.0	3.51	3.4274 (1.75)	3.2208 (6.14)	3.4777 (0.69)
4.0	2.59	2.8980 (−8.86)	2.6901 (−2.88)	2.8726 (−8.13)
6.0	1.92	2.0464 (−4.91)	1.9381 (−0.70)	1.9997 (−3.10)
8.0	1.29	1.4026 (−6.51)	1.3608 (−4.09)	1.3617 (−4.15)
10.0	0.92	0.9385 (−1.50)	0.9294 (−0.76)	0.9083 (0.95)
12.0	0.63	0.6176 (1.47)	0.6228 (0.85)	0.5977 (3.83)
14.0	0.39	0.4020 (−2.30)	0.4128 (−4.36)	0.3900 (−0.01)
16.0	0.27	0.2600 (2.77)	0.2721 (−0.59)	0.2533 (4.60)

[a] Simulated plasma levels containing 10% random noise. [b] Weighted residuals are given in parentheses ($\times 10^2$).

ability of the estimated parameters and of the efficiency of the estimation procedure. As can be seen in Table III, the errors associated with either the spline or the LEASQ results are in the same range as the expected error. Compared to the errors of the LEASQ solutions, the expected *ASE* values were greater but the expected *WSS* was smaller. The first observation suggests that the true parameters probably are irretrievable from corrupted input data. The second observation indicates that further improvement of the parameter estimates can be made by applying superior numerical algorithms. This conclusion is manifested in Table VI where the predicted and the observed (input) data are compared.

Conclusions—Two numerical examples have been described to illustrate the proposed method in the estimation of pharmacokinetic parameters. For a given model and weighting scheme, the reliability of the estimates is dependent on data accuracy as well as on the numerical algorithms employed. Obviously, when experimental errors are large, meaningful estimates are difficult, irrespective of algorithmic sophistication.

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Impurities in Drugs III: Trihexyphenidyl

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Received January 17, 1979, from the *Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada.* Accepted for publication March 16, 1979.

Abstract □ Two lots of trihexyphenidyl hydrochloride raw material, one lot of elixir, and 10 lots of tablets were examined for impurities by TLC. Impurities found were 1-phenyl-2-propenone, 3-piperidinopropiophenone, and 3-aminopropiophenone. Not all impurities were present in all lots, and none exceeded 1.9% of the label drug claim. Impurities were identified by mass spectrometry and by comparison of TLC R_f values and GLC retention times to those of synthesized specimens of the impurities.

Keyphrases □ Trihexyphenidyl—analysis, TLC, impurities in tablets and elixir □ Trihexyphenidyl, derivatives—1-phenyl-2-propenone, 3-piperidinopropiophenone, 3-aminopropiophenone, TLC analysis, impurities in tablets and elixir □ Drug impurities—trihexyphenidyl, tablets and elixir, TLC analysis

Impurities in drug raw materials and formulations may be intermediates or by-products of the synthetic process, products of degradation or drug–excipient interaction, or the result of contamination. The nature of impurities may depend on the synthetic route, the reagent purity, and the excipient quality. To obtain a good perspective of potential impurities, raw materials and formulations from as many sources as possible should be examined (1–4). This paper describes the impurities found in trihexyphenidyl (I) raw material and tablet and elixir products.

Trihexyphenidyl was synthesized first (5) by the addition of cyclohexylmagnesium bromide to 3-piperidinopropiophenone (II), obtained by the Mannich reaction with acetophenone (III), formaldehyde, and piperidine hydrochloride in acidic medium. Trihexyphenidyl hydrochloride raw material and tablets are official in the USP (6) and BP as benzhexol (7). An elixir is official in the USP only. The only impurity specification is that in the BP for 3-piperidinopropiophenone in drug raw material.

EXPERIMENTAL

Materials—All drugs and formulations were obtained from the

0022-3549/79/0900-1124$01.00/0