Chapter 857

Confidence Intervals for Michaelis-Menten Parameters

Introduction

This routine calculates the sample size necessary to achieve specified widths of the confidence intervals of the parameters of the Michaelis-Menten equation at a stated confidence level. The analysis assumes that the parameters are estimated using maximum likelihood and that the error variance is proportional to the independent variable.

Caution: This procedure assumes that parameter estimates of the future sample will be the same as planning estimates that are specified. If actual estimates are very different from those specified when running this procedure, the interval width may be narrower or wider than specified.

Technical Details

Michaelis-Menten Equation

The formulas used here are found in Raaijmakers (1987). The Michaelis-Menten equation is a well-known model of enzyme kinetics. It is a special arrangement of a two-parameter rectangular hyperbola. The mathematical model is

$$V = \frac{C(Vmax)}{C + Km}$$

where *V* is the dependent variable, *C* is the independent variable, and *Vmax and Km* are parameters to be estimated. In enzyme kinetics, *V* is the velocity (rate) of an enzyme reaction and *C* is the substrate concentration. *Vmax* and *Km* have simple physical interpretations. *Vmax* is the maximum velocity and serves as a horizontal asymptote. *Km* is the value of *C* the results a velocity of *Vmax*/2. It is known as the Michaelis constant or ED50.

Estimation

Although several methods have been proposed to estimate the parameters of the Michaelis-Menten equation from a set of data consisting of concentrations and corresponding rates, we will use the method of maximum likelihood because it leads to simple, analytical formulae for the parameters as well as large-sample confidence intervals.

The nonlinear regression model associated with this equation is

$$V = \frac{C(Vmax)}{C + Km} + \varepsilon$$

where ε represents normally distributed errors with zero mean and variance σ^2. However, this assumption of a constant absolute error is not appropriate in most situations. It is usually more appropriate to assume that σ is proportional to the mean value at each value of C. This leads to the following statistical model

$$V = \frac{C(Vmax)}{C + Km} + \left(\frac{C(Vmax)}{C + Km}\right)\varepsilon$$

If we let X equal V/C, Raaijmakers (1987) provided the following estimates of the parameters and their variances.

$$\widehat{Vmax} = \bar{V} + \widehat{Km}\,\bar{X}$$

$$\widehat{Km} = \frac{\bar{X}S_{VV} - \bar{V}S_{XV}}{\bar{V}S_{XX} - \bar{X}S_{XV}}$$

where S_{VV}, S_{XV}, and S_{XX} are the sum of squares and cross products of the deviations $\mathrm{V} - \bar{\mathrm{V}}$ and $\mathrm{X} - \bar{\mathrm{X}}$. $\bar{\mathrm{V}}$ and $\bar{\mathrm{X}}$ are the sample means of the corresponding variables.

An unbiased estimate of the error variance is given by

$$\hat{\sigma}^2 = \frac{S_{VV} + 2\widehat{Km}S_{XV} + \widehat{Km}^2 S_{XX}}{N - 2}$$

where *N* is the sample size.

The large-sample variances of these estimates are given by

$$\mathrm{var}\left(\widehat{Km}\right) \approx \frac{\sigma^2}{\left(1 + 2\sigma^2/\widehat{Vmax}^2\right)\sum_{i=1}^{N}(U_i - \bar{U})^2}$$

$$\mathrm{var}\left(\widehat{Vmax}\right) \approx \frac{\sigma^2}{N} + \bar{U}^2\mathrm{var}\left(\widehat{Km}\right)$$

where

$$U_i = \frac{Vmax}{C_i + Km}$$

Finally, confidence intervals can be calculated for these estimates based on the normality of the estimates. The 100(1 – α)% confidence interval for *Km* is

$$\widehat{Km} \pm \mathrm{z}_{1-\alpha/2}\sqrt{\mathrm{var}(Km)}$$

The 100(1 – α)% confidence interval for *Vmax* is

$$\widehat{Vmax} \pm \mathrm{z}_{1-\alpha/2}\sqrt{\mathrm{var}\left(\widehat{Vmax}\right)}$$

The widths of these intervals are easily determined.

A close inspection of these formulas will show that they depend on the experimental design. That is, the *C* values. So, the sample size formulae require the specification of a specific design.

Confidence Level

The confidence level, 1 – α, has the following interpretation. If thousands of samples of n items are drawn from a population using simple random sampling and a confidence interval is calculated for each sample, the proportion of those intervals that will include the true population slope is 1 – α.

Example 1 – Calculating Sample Size

Suppose a study is planned in which a researcher wishes to construct 95% confidence intervals for Vmax and Km. Previous studies have found Vmax to range from 20 to 30 and Km to be about 10. The confidence level is set at 0.95. The standard deviation of the residuals estimate, based on the MSE from a similar study, ranges from 10 to 40. The sample size needs to be large enough so that the width of the Vmax confidence interval is 5.

The design is to set the number of C values to 5. The individual C values are 1, 4, 16, 64, and 256.

Setup

If the procedure window is not already open, use the PASS Home window to open it. The parameters for this example are listed below and are stored in the **Example 1** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Design Tab

Solve For ....**Sample Size using Vmax**
Confidence Level **0.95**
Number of Unique C Values **5**
Allocation of N to C Values ....**Equal sample sizes for all C values (n = n1 = n2 = …)**
Vmax (Maximal Velocity) **20 30**
Vmax Confidence Interval Width...................**5**
Km (Michaelis Constant)...............................**10**
σ (Error Standard Deviation).........................**10 20 30 40**
C1 ....**1**
C2 ....**4**
C3 ....**16**
C4 ....**64**
C5 ....**256**

Output

Click the Calculate button to perform the calculations and generate the following output.

Numeric Reports

Numeric Results

Solve For: Sample Size using Vmax
C Values: 1, 4, 16, 64, 256

Row	Confidence Level	Total Sample Size N	Error Standard Deviation σ	Maximal Velocity (Vmax) Vmax	SE(Vmax)	CI Width	Michaelis Constant (Km) Km	SE(Km)	CI Width
1	0.95	135	10	20	1.261	4.943	10	1.056	4.141
2	0.95	390	20	20	1.270	4.979	10	0.879	3.445
3	0.95	730	30	20	1.272	4.987	10	0.712	2.790
4	0.95	1175	40	20	1.274	4.992	10	0.585	2.292
5	0.95	150	10	30	1.267	4.965	10	0.740	2.901
6	0.95	470	20	30	1.275	4.998	10	0.673	2.637
7	0.95	875	30	30	1.272	4.986	10	0.587	2.300
8	0.95	1355	40	30	1.275	4.999	10	0.510	2.000

Michaelis-Menten Equation with Variance Proportional to Substrate Concentration

Model V = [(Vmax × C) / (Km + C)] + [(Vmax × C) / (Km + C)] × ε
Dependent Variable V is the Reaction Rate (Velocity).
Independent Variable C is the Substrate Concentration.
Error Variable ε is the Residual. The model assumes that ε ~ N(0, σ²).

Confidence Level The proportion of confidence intervals (constructed with this same confidence level) that would contain the true value of the estimated parameters: Vmax or Km.
N The total sample size of the experiment.
σ The standard deviation of ε when (Vmax C) / (Km + C) = 1.
Vmax A planning estimate of the maximum reaction rate (V).
SE(Vmax) The standard error of the maximum likelihood estimate of Vmax.
Vmax CI Width The width of the confidence interval of Vmax.
Km A planning estimate of the Michaelis constant. This is the C value that yields a V of Vmax/2. It is sometimes called ED50.
SE(Km) The standard error of the maximum likelihood estimate of Km.
Km CI Width The width of the confidence interval of Km.

Summary Statements

A single-group Michaelis-Menten equation design will be used to obtain a two-sided 95% confidence interval for each of the two Michaelis-Menten Parameters: the maximal velocity (Vmax) and the Michaelis Constant (Km). The sample-estimated Vmax is assumed to be 20, the sample-estimated Km is assumed to be 10, and the error standard deviation (σ) is assumed to be 10. To produce a confidence interval for Vmax with a width of no more than 5, 135 total subjects will be needed. The resulting confidence interval width for Km, with 135 total subjects, is 4.141. The experimental design consists of the C values 1, 4, 16, 64, 256, with corresponding sample sizes of 27, 27, 27, 27, 27, respectively.

Design Details for Row 1

C Level	C	n	Pct of N
C1	1	27	20
C2	4	27	20
C3	16	27	20
C4	64	27	20
C5	256	27	20

.
.
.

Dropout-Inflated Sample Size

Group	Dropout Rate	Sample Size n	Dropout-Inflated Enrollment Sample Size n'	Expected Number of Dropouts D
C1 - C5	20%	27	34	7
Total		135	170	35
C1 - C5	20%	78	98	20
Total		390	490	100
C1 - C5	20%	146	183	37
Total		730	915	185
C1 - C5	20%	235	294	59
Total		1175	1470	295
C1 - C5	20%	30	38	8
Total		150	190	40
C1 - C5	20%	94	118	24
Total		470	590	120
C1 - C5	20%	175	219	44
Total		875	1095	220
C1 - C5	20%	271	339	68
Total		1355	1695	340

Group — Lists the group numbers.
Dropout Rate — The percentage of subjects (or items) that are expected to be lost at random during the course of the study and for whom no response data will be collected (i.e., will be treated as "missing"). Abbreviated as DR.
n — The evaluable sample size for each group at which the confidence interval is computed. If n subjects are evaluated out of the n' subjects that are enrolled in the study, the design will achieve the stated confidence interval.
n' — The number of subjects that should be enrolled in each group in order to obtain n evaluable subjects, based on the assumed dropout rate. After solving for n, n' is calculated by inflating n using the formula n' = n / (1 - DR), with n' always rounded up. (See Julious, S.A. (2010) pages 52-53, or Chow, S.C., Shao, J., Wang, H., and Lokhnygina, Y. (2018) pages 32-33.)
D — The expected number of dropouts in each group. D = n' - n.

Dropout Summary Statements

Anticipating a 20% dropout rate, group sizes of 34, 34, 34, 34, and 34 subjects should be enrolled to obtain final group sample sizes of 27, 27, 27, 27, and 27 subjects.

References

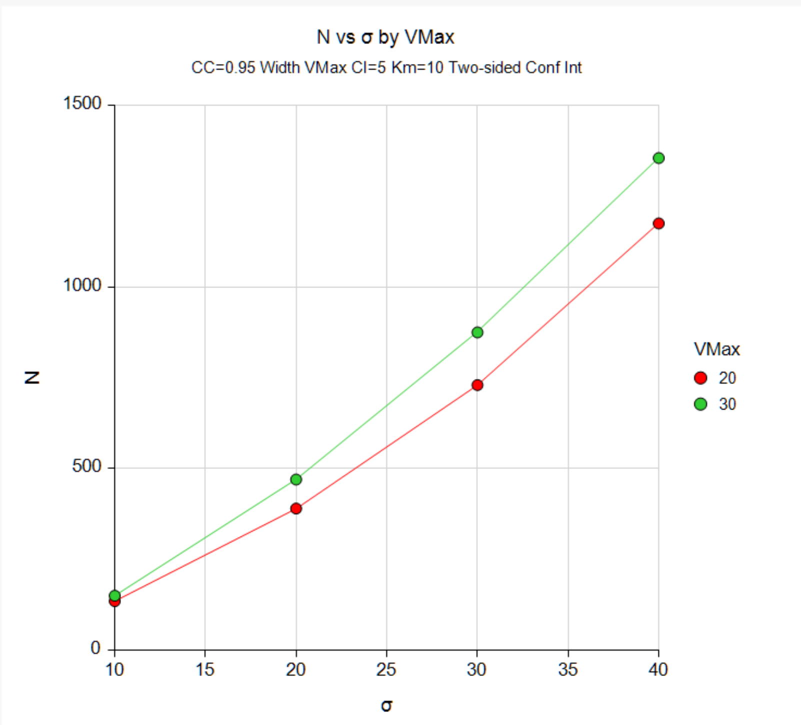
Raaijmakers, Jeroen G.W. 1987. 'Statistical Analysis of the Michaelis-Menten Equation'. Biometrics. Vol. 43, No. 4, 793-803.

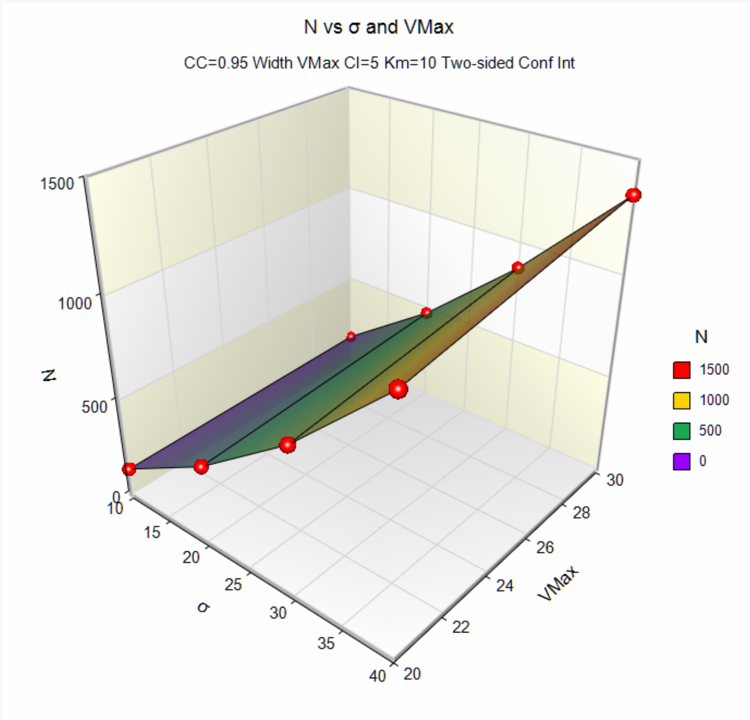
This report shows the calculated sample size for each of the scenarios.

The Design Details report shows how the 135 subjects are allocated to the various C values. In the first case, 27 subjects are allocated to each of the five C values.

Plots Section

Plots





These plots show how the necessary sample size changes for variance values of the error standard deviation and Vmax.

Example 2 – Validation using Raaijmakers (1987)

Raaijmakers (1987) page 798 gives an in which a researcher wishes to construct 95% confidence intervals for Vmax and Km. The design is to set the number of C values to 5. The individual C values are 1, 4, 16, 64, and 256. Previous studies have found Vmax be 25 and Km to be 10. The confidence level is set at 0.95. The standard deviation of the residuals is estimated at 6.25. The sample sizes of 5, 10, and 20 result in SE(Vmax) values of 4.444, 3.143, and 2.222 and SE(Km) of 3.169, 2.241, and 1.585. The confidence interval widths are easily calculated from these standard errors.

Setup

If the procedure window is not already open, use the PASS Home window to open it. The parameters for this example are listed below and are stored in the **Example 2** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Design Tab

Solve For	**CI Widths of Vmax and Km**
Confidence Level	**0.95**
Number of Unique C Values	**5**
Allocation of N to C Values	**Equal sample sizes for all C values (n = n1 = n2 = …)**
N (Sample Size for All C Values)	**1 2 4**
Vmax (Maximal Velocity)	**25**
Km (Michaelis Constant)	**10**
σ (Error Standard Deviation)	**6.25**
C1	**1**
C2	**4**
C3	**16**
C4	**64**
C5	**256**

Output

Click the Calculate button to perform the calculations and generate the following output.

Numeric Results

Solve For: CI Widths of Vmax and Km
C Values: 1, 4, 16, 64, 256

		Total	Error	Maximal Velocity (Vmax)			Michaelis Constant (Km)		
	Confidence	Sample Size	Standard Deviation						
Row	Level	N	σ	Vmax	SE(Vmax)	CI Width	Km	SE(Km)	CI Width
1	0.95	5	6.25	25	4.444	17.422	10	3.169	12.423
2	0.95	10	6.25	25	3.143	12.319	10	2.241	8.784
3	0.95	20	6.25	25	2.222	8.711	10	1.585	6.211

Michaelis-Menten Equation with Variance Proportional to Substrate Concentration

Model	V = [(Vmax × C) / (Km + C)] + [(Vmax × C) / (Km + C)] × ε
Dependent Variable	V is the Reaction Rate (Velocity).
Independent Variable	C is the Substrate Concentration.
Error Variable	ε is the Residual. The model assumes that ε ~ N(0, σ²).

PASS matches these standard errors exactly. Note that the confidence interval width is 4.444 x 1.96 x 2 = 17.42 which matches **PASS's** result within rounding.