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DECUS NO. FOCAL8-198

TITLE MICHAELIS-MENTEN KINETICS

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SOURCE LANGUAGE FOCAL '69

MICHAELIS-MENTEN KINETICS

ABSTRACT

A FOCAL program to provide maximum likelihood estimates of the parameters VMAX and KM of the Michaelis-Menten equation. Standard errors and both 95 and 99% confidence limits of the parameters are also provided. Fitted data points and the reciprocals of the estimates are printed out for graphical purposes.

REQUIREMENTS

Hardware - PDP-8 with 8K of memory, teletype

Software - FOCAL 1969 (DEC-08-AJAE-PB)
- 8K, 4 word overlay (DEC-08-AJ1E-PB)

Locations - FOCAL text occupies locations 100-4705 of FIELD-1

USAGE

Loading - 1. Load FOCAL 1969 (DEC-08-AJAE-PB) with the BINARY LOADER - press continue once.

2. Start up FOCAL (SA 0200) and delete the extended functions by answering NO to both extended function queries.

3. Load the 8K and 4 word overlays (DEC-08-AJ1E-PB) with the BINARY LOADER - press continue once.

4. Delete : and = with the following patch

ADDR	FROM	TO	
1217 /	4551	7600	(deletes :)
6002 /	4551	7600	(deletes =)

5. Start up FOCAL

6. Load the Michaelis-Menten Kinetics Program 'HYPERB' from FOCAL

Test data - To execute the program with the set of test data provided, type GOTO 9.1. The data will first be listed, then the normal output. This data is from Wilkinson (1) and is the data used in the Yale Computer Center FORTRAN program 'HYPERB' (2). This data was also used as test data in a paper by Bliss and James (3) upon which the statistical procedure is based.

Sample data - To execute the program with your own data, type GO. The program will ask for the number of data points N, then ask for the data S(1), V(1), S(2), V(2), ..., S(N), V(N). The space bar is to be used to enter all data. The program is preset to run to convergence or 40 iterations. If convergence fails a message is typed out. As the program uses K = 0 as a preliminary estimate and a sufficient no. of iterations is provided for convergence with even the most variable data, a failure to converge most likely indicates a divergence. This means that the curve relating S and V curves upward with increasing increments in S rather than heading toward a plateau (VMAX). In this event the data does not follow Michaelis-Menten kinetics. The program does however allow you the option of printing out the iteration table (to verify that divergence is taking place). A negative K value may then be entered. If convergence then succeeds both VMAX and K will be negative - a result which is uninterpretable in terms of Michaelis-Menten kinetics. One of the confidence limits may however be positive and this may be of some use.

A student t-table is provided internally by the program for up to 20 degrees of freedom. Only in the event that the degrees of freedom exceeds 20 (23 or more data points) will the program request t-table values.

A printout of the estimates, their standard errors, and both 95 and 99% confidence limits is then provided. Reciprocals and their limits are also provided in the event that one of the other (Lineweaver-Burk) straight line plots is desired. A table of variances and standard errors is also printed out.

Errors in usage - Error messages may occur if divergence is taking place. In that event, the program should then be restarted with a GOTO 2.42. If a data value is incorrectly entered, the program should be restarted (Contr/C, GO).

RESTRICTIONS

The program will accept a maximum of 30 data points. A minimum of 3 data points is required.

DISCUSSION

Program source - The program is derived from the Yale Computer Center FORTRAN program 'HYPERB' by K.R. Hanson, R. Ling, and E. Havir (2). The FOCAL program differs in that it is set at the Michaelis-Menten option, it starts with a preliminary estimate for K of zero, has a built in t-table, has the output organized in a different form and has eliminated a mandatory listing of the iteration table and some of the output relating to reciprocals of confidence limits. It also does not have a graphical output option. As far as possible it uses the same variable identifiers and structure.

Statistics source - The statistical procedure is based on that of Bliss and James (3).

REFERENCES

1. Wilkinson, G.N. Statistical Estimations in enzyme kinetics. Biochem, J. 80:324-32, 1961.

2. Hanson, K.R., Ling, R. and Havir, E. Yale Computer Center FORTRAN program 'HYPERB'.

3. Bliss, C.I. and James, A.T. Fitting the Rectangular Hyperbola. Biometrics 22:573-602, 1966.

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C-8K FOCAL @1969

01.01 C***** HYPERB *****    OCT 13 1971
01.02 C-----A FOCAL ADAPTATION OF THE YALE COMPUTER CENTER FORTRAN
01.03 C-----    PROGRAM 'HYPERB' USED FOR FITTING DATA TO THE
01.04 C-----        MICHAELIS-MENTEN EQUATION
01.05 C-----            ...STANLEY R. VIVIAN...
01.10 E
01.20 T !!"            MICHAELIS-MENTEN REGRESSION
01.25 A !!"N:"N
01.26 I (N-3)1.28;I (N-31)1.3
01.27 T !!"TOO MANY DATA POINTS - MAXIMUM N IS 30";G 5.5
01.28 T !!"TOO FEW DATA POINTS - MINIMUM N IS 3";G 5.5
01.30 T !"    S         V";F I=1,N;A !":"XP(I)," :"Y(I)
01.40 S D=DO;S IT=0
01.50 S X=0;S XS=0;S Y=0;S X2=0;S Y2=0;S S2=0;S XX=0;S XY=0;S SY=0
01.60 F I=1,N;S XI=XP(I)/(XP(I)+D);S XJ=XI/(XP(I)+D);D 1.7;D 1.9;D 1.92
01.65 G 2.1
01.70 S X=X+XI;S XS=XS+XJ;S Y=Y+Y(I)
01.90 S Y2=Y2+Y(I)↑2;S S2=S2+XJ↑2;S XX=XX+XI*XJ;S X2=X2+XI↑2
01.92 S XY=XY+XI*Y(I);S SY=SY+XJ*Y(I)

02.10 S B1=XY*S2-SY*XX;S B2=SY*X2-XY*XX;I (B1)2.12,2.4,2.12
02.12 S BR=B2/B1
02.15 I (SW-1)2.3,2.2,2.3
02.20 T !%2,IT,%5.1,D,X,XS,X2,XX,S2,XY,SY," "%8.06,BR
02.25 I (IT-15)2.3;G 2.4
02.30 I (FABS(BR)-.000001)2.6,2.6;I (IT-40)2.5,2.5;T "";C--- 10 BELLS
02.40 I (SW-1)2.42;S SW=SW+1;G 2.47
02.42 T !!"FAILURE AT CONVERGENCE - K AND VMAX ARE NEGATIVE
02.44 A !"DO YOU WANT AN ITERATION TABLE? "IT
02.46 I (IT-0N)2.49,2.47;I (IT-0Y)2.49,2.48,2.49
02.47 A !"ENTER A NEGATIVE K OR TYPE '/' TO TERMINATE: "DO;I (DO)1.4;D 5
02.48 S SW=SW+1;D 2.52;D 2.54;G 1.4
02.49 T !"PLEASE ANSWER 'Y' OR 'N'";G 2.44
02.50 S D=D-BR;S IT=IT+1;G 1.5
02.52 T !!"            ITERATION TABLE",!"       K
02.54 T "SUMX   SUMX*  SUMXX SUMXX* SUMX*X* SUMXY SUMX*Y   B-RATIO
02.60 S DS=X2*S2-XX↑2;S B1=B1/DS;S B2=B2/DS;S BC=XY/X2;S NF=N-2
02.70 S SS=(Y2-B1*XY-B2*SY)/NF;S S=FSQT(SS);S SC=(Y2-BC*XY)/NF
02.80 S C1=S2/DS;S C2=-XX/DS;S C"=X2/DS;S VA=SS*C1;S SE=FSQT(VA)
02.90 S RV=1/VA;S RB=1/B1;S VR=(SS/B1↑2)*(C"-2*C2*BR);S SV=FSQT(VR)

03.10 S RR=1/VR;S VB=VA/B1↑4;S SR=FSQT(VB);S R1=1/VB
03.20 S VD=(SS/B1↑4)*(C"-4*C2*BR);S SB=FSQT(VD);S RD=1/VD;S R'=1/D
03.30 S DO=D/B1;S BO=B1/D
03.40 T !!"                        PLOT DATA
03.50 T !"      S          V          X=S/(S+K)   V(CALC)        V-VCALC
03.60 F I=1,N;S XI=XP(I)/(XP(I)+D);S YY=XI*B1;S DI=Y(I)-YY;D 3.8
03.70 G 4.05
03.80 T !%8.05,XP(I),Y(I),"    "XI,YY,"   "DI

04.05 D 7
04.10 T !!!"MAXIMUM LIKELIHOOD                   CONFIDENCE INTERVAL",!
04.20 T "   ESTIMATE                -SE+             95%              99%
04.30 T !!"VMAX   ="%6.05,B1;F J=1,3;D 6.1
04.32 T !"1/VMAX="RB;F J=1,3;T "    "1/U(J),1/L(J)
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04.34 T !!"K       ="D;F J=1,3;D 6.3;D 6.35
04.35 T !"1/K    =",R';F J=1,3;T "    "1/U(J),1/L(J)
04.36 T !!"K/VMAX="DO;F J=1,3;D 6.6;D 6.7
04.38 T !"VMAX/K="BO;F J=1,3;T "    "1/U(J),1/L(J)
04.40 T !!!!"        ANALYSIS OF VARIANCE
04.50 T !"VAR OF V =",SS,"     SE ="FSQT(SS)
04.60 T !!"VARIANCE      VMAX ="VA,"  SE ="SE,"   1/VAR ="RV
04.70 T !"ASYM VAR  1/VMAX ="VB,"  SE ="SR,"  1/VAR ="R1
04.80 T !"ASYM VAR  K        ="VR,"  SE ="SV,"  1/VAR ="RR
04.90 T !"ASYM VAR  K/VMAX ="VD,"  SE ="SB,"  1/VAR ="RD

05.50 T !!!!!
05.60 QUIT

06.05 T !%3,J,%5.1
06.10 S S=T(J)*SE;S L(J)=B1-S;S U(J)=B1+S;T "    "L(J),U(J)
06.30 S C=B1↑2/(B1↑2-C1*SS*T(J)↑2);S K=(C-1)*C2/C1;S XD=D-C*BR-K
06.35 S S=(C-1)*(C*(BR-C2/C1)↑2+1/C1/S2);I (0-S)6.4;G 6.36
06.36 S L(J)=99E99;S U(J)=L(J);T "    [- INFINITY +] "
06.40 S S=FSQT(S);S L(J)=XD-S;S U(J)=XD+S;D 6.5
06.50 T "   "L(J),U(J)
06.60 S C=B1↑2-4*C1*SS*T(J)↑2;S W=DO+(B1*B2-2*C2*SS*T(J)↑2)/B1/C
06.70 S S=SS*(C*C"-4*C2*(B1*B2-C2*SS*T(J)↑2));I (0-S)6.75;D 6.36
06.75 S S=T(J)*FSQT(S)/B1/C;D 6.8
06.77 T " [ -INFINITY+ ] "
06.80 S L(J)=W-S;S U(J)=W+S;T "    "L(J),U(J)

07.01 C------STUDENT'T VALUE TABLE (1-20) DF
07.10 S V=NF;D 8;S T(1)=1;S T(2)=A;S T(3)=B

08.10 I (1-V)8.11;S A=12.706;S B=63.657;R
08.11 I (2-V)8.12;S A=4.303;S B=9.925;R
08.12 I (3-V)8.13;S A=3.182;S B=5.841;R
08.13 I (4-V)8.14;S A=2.776;S B=4.604;R
08.14 I (5-V)8.15;S A=2.571;S B=4.032;R
08.15 I (6-V)8.16;S A=2.447;S B=3.707;R
08.16 I (7-V)8.17;S A=2.365;S B=3.499;R
08.17 I (8-V)8.18;S A=2.306;S B=3.355;R
08.18 I (9-V)8.19;S A=2.262;S B=3.25;R
08.19 I (10-V)8.2;S A=2.228;S B=3.169;R
08.20 I (11-V)8.21;S A=2.201;S B=3.106;R
08.21 I (12-V)8.22;S A=2.179;S B=3.055;R
08.22 I (13-V)8.23;S A=2.16;S B=3.012;R
08.23 I (14-V)8.24;S A=2.145;S B=2.977;R
08.24 I (15-V)8.25;S A=2.131;S B=2.947;R
08.25 I (16-V)8.26;S A=2.12;S B=2.921;R
08.26 I (17-V)8.27;S A=2.11;S B=2.898;R
08.27 I (18-V)8.28;S A=2.101;S B=2.878;R
08.28 I (19-V)8.29;S A=2.093;S B=2.861;R
08.29 I (20-V)8.3;S A=2.086;S B=2.845;R
08.30 T !!!"ENTER 2-SIDED STUDENT T VALUES FOR"%3,V," DEGREES OF FREEDOM
08.32 A !"P=.05: "A,"  P=.01: "B

09.01 C-----TEST DATA
09.10 D 1.1;D 1.2;T !!"** TEST DATA **";S N=6;T !!"N:"%1,N,!"    S       V"
09.20 S XP(1)=.138;S Y(1)=.148;S XP(2)=.220;S Y(2)=.171
09.30 S XP(3)=.291;S Y(3)=.234;S XP(4)=.560;S Y(4)=.324
09.40 S XP(5)=.766;S Y(5)=.390;S XP(6)=1.46;S Y(6)=.493
09.50 F I=1,N;T !":"%4.03,XP(I)," :"Y(I)
09.60 G 1.4
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 GOTO 9.1

          MICHAELIS-MENTEN REGRESSION

** TEST DATA **

N: 6
    S       V
: 0.138 : 0.148
: 0.220 : 0.171
: 0.291 : 0.234
: 0.560 : 0.324
: 0.766 : 0.390
: 1.460 : 0.493

                    PLOT DATA
     S          V          X=S/(S+K)   V(CALC)        V-VCALC
   0.13800    0.14800       0.18787   0.12971        0.01830
   0.22000    0.17100       0.26943   0.18601    -   0.01501
   0.29100    0.23400       0.32787   0.22636        0.00764
   0.56000    0.32400       0.48420   0.33429    -   0.01029
   0.76600    0.39000       0.56218   0.38813        0.00187
   1.46000    0.49300       0.70993   0.49013        0.00287


MAXIMUM LIKELIHOOD                          CONFIDENCE INTERVAL
   ESTIMATE                  -SE+                 95%                  99%

VMAX   = 0.69040     0.65358 0.72723     0.58818 0.79263     0.52086 0.85994
1/VMAX= 1.44844     1.37509 1.53005     1.26163 1.70018     1.16287 1.91991

K      = 0.59655     0.53156 0.66844     0.43016 0.81716     0.34082 1.00750
1/K    = 1.67631     1.49602 1.88127     1.22375 2.32475     0.99255 2.93413

K/VMAX= 0.86406     0.77420 0.97409     0.64883 1.24768     0.55040 1.73460
VMAX/K= 1.15733     1.02660 1.29166     0.80149 1.54123     0.57650 1.81686


     ANALYSIS OF VARIANCE
VAR OF V = 0.00019     SE = 0.01357

VARIANCE     VMAX = 0.00136   SE = 0.03683   1/VAR = 737.435
ASYM VAR   1/VMAX = 0.00597   SE = 0.07726   1/VAR = 167.543
ASYM VAR   K      = 0.00466   SE = 0.06826   1/VAR = 214.637
ASYM VAR   K/VMAX = 0.00978   SE = 0.09887   1/VAR = 102.307


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