OPTIMUM DESIGNS FOR PARAMETER ESTIMATION AND MODEL DISCRIMINATION IN ENZYME INHIBITION STUDIES

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ENZYME KINETICS

In a typical enzyme kinetics reaction enzymes bind substrates and turn them into products:

$$S + E \longleftrightarrow ES \rightarrow E + P,$$

where S, E and P denote substrate, enzyme and product. The reaction rate is represented by the standard Michaelis-Menten model

$$v = \frac{V[S]}{K_m + [S]}$$

where $[S]$ is the concentration of the substrate and V and K_m are the model parameters:

- V denotes the maximum velocity of the enzyme,
- K_m is the value of $[S]$ at which half of the maximum velocity V is reached.

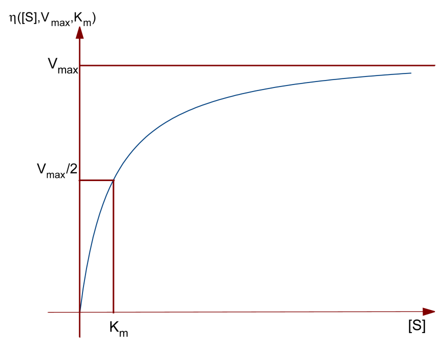


Figure 1: Michaelis-Menten Model for enzyme kinetics

ENZYME KINETICS WITH INHIBITION

- Relevant when Drug-Drug Interaction is possible.
- Co-administration of a drug with an inhibitor of the enzyme that metabolizes it, can lead to a reduction in the metabolism of a substrate and potentially cause an adverse drug reaction as a result of the raised plasma concentration of the drug.
- There are several types of inhibition. For example:

Competitive Inhibition

$$\begin{array}{cccccc} & & EI & & & \\ & & \updownarrow K_{ic} & & & \\ S & + & E & \longleftrightarrow & ES & \longrightarrow E + P. \\ & & + & & & \\ & & I & & & \end{array}$$

Non-Competitive Inhibition

$$\begin{array}{cccccc} & & & & EIS & \longrightarrow EI + P \\ & & & & \updownarrow K_{iu} & \\ S & + & E & \longleftrightarrow & ES & \longrightarrow E + P. \\ & & & & + & \\ & & & & I & \end{array}$$

Mixed Inhibition

$$\begin{array}{cccccc} & & EI & & EIS & \longrightarrow EI + P \\ & & \updownarrow K_{ic} & & \updownarrow K_{iu} & \\ S & + & E & \longleftrightarrow & ES & \longrightarrow E + P. \\ & & + & & + & \\ & & I & & I & \end{array}$$

ENZYME KINETICS WITH COMPETITIVE INHIBITION

Inhibitor and substrate binding are mutually exclusive. The velocity equation is:

$$v = \frac{V[S]}{K_m\left(1 + \frac{[I]}{K_{ic}}\right) + [S]}$$

where K_{ic} is the inhibition constant.

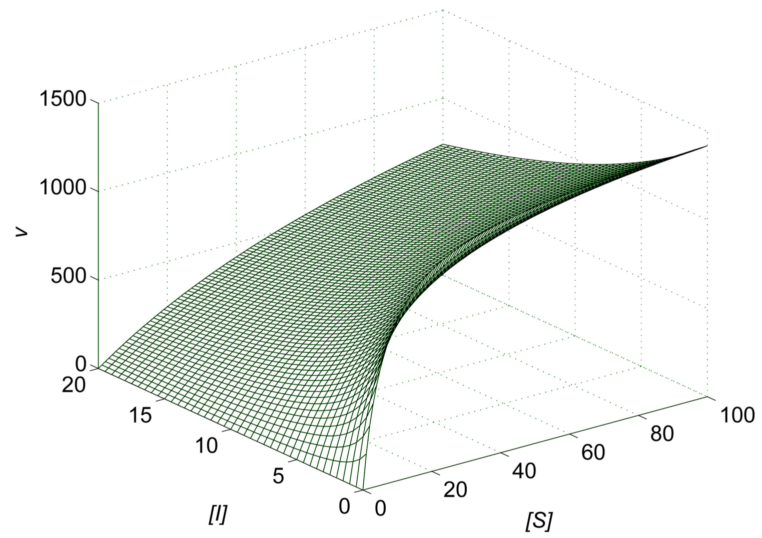


Figure 2: Velocity of the competitive inhibition enzyme kinetics

DESIGN PROBLEM

- Efficient estimation of all the model parameters

NOTATION

Design: probability measure on a finite number of points x_i,

$$\xi = \begin{Bmatrix} x_1 & \dots & x_s \\ w_1 & \dots & w_s \end{Bmatrix}, \qquad \sum_{i=1}^{s} w_j = 1,\ w_i > 0$$

Statistical Model:

$$y_i = \eta(x_i, \boldsymbol{\vartheta}) + \varepsilon_i, \qquad \varepsilon_i \underset{iid}{\sim} \mathcal{N}(0, \sigma^2),$$

where x_i are the support points of design ξ and $\boldsymbol{\vartheta}$ denotes a p-dimensional vector of parameters.

D-OPTIMUM DESIGN - COMPETITIVE KINETICS

D-optimum design $\xi_D^\star$ maximizes the determinant of the information matrix M at a prior value of the parameter vector $\boldsymbol{\vartheta}$, that is

$$\xi_D^\star = \max_{\xi \in \Xi} \det M(\xi, \boldsymbol{\vartheta}^0)$$

Here we have (Bogacka et al, 2011)

$$x_i = ([S]_i, [I]_i), \ \ [S]_{\min} \le [S]_i \le [S]_{\max}, \ \ [I]_{\min} \le [I]_i \le [I]_{\max}.$$

- D-optimum design form:

$$\xi_D^\star = \begin{Bmatrix} ([S]_{\max}, [I]_{\min}) & ([S]_2, [I]_{\min}) & ([S]_3, [I]_3) \\ \frac{1}{3} & \frac{1}{3} & \frac{1}{3} \end{Bmatrix}$$

where $[S]_2, [S]_3, [I]_3$ are functions of $\boldsymbol{\vartheta} = (V, K_m, K_{ic})$,

$$[S]_2 = \max\left\{ [S]_{\min}, \ \frac{[S]_{\max} K_m (K_{ic} + [I]_{\min})}{2K_m K_{ic} + 2K_m [I]_{\min} + [S]_{\max} K_{ic}} \right\}$$

$$[S]_3 = \max\left\{ [S]_{\min}, \ \min\left\{ \frac{K_m (K_{ic} + [I]_{\max})}{K_{ic}}, \ [S]_{\max} \right\} \right\}$$

$$[I]_3 = \min\left\{ \frac{2K_m [I]_{\min} + [S]_{\max} K_{ic} + K_m K_{ic}}{K_m}, \ [I]_{\max} \right\}$$

- For the prior parameter values $(V^0, K_m^0, K_{ic}^0) = (7.30, 4.39, 2.58)$ and $0 \le [S]_i \le 60$, $0 \le [I]_i \le 30$, we obtain

$$\xi_D^\star = \begin{Bmatrix} (30,\ 0) & (3.39,\ 0) & (30,\ 20.24) \\ \frac{1}{3} & \frac{1}{3} & \frac{1}{3} \end{Bmatrix}$$

PARAMETER SENSITIVITIES

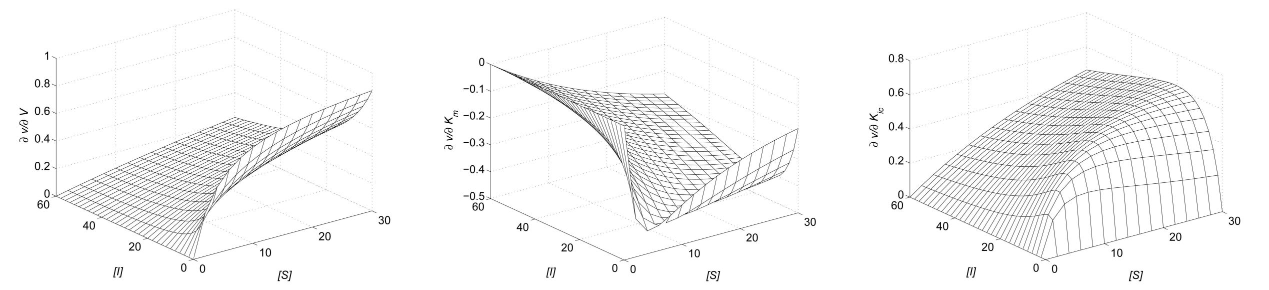


Figure 3: Derivatives of the model function with respect to the parameters are informative for finding optimum designs: regions of their extrema indicate high variability of model prediction.

EQUIVALENCE THEOREM AND DESIGN POINTS

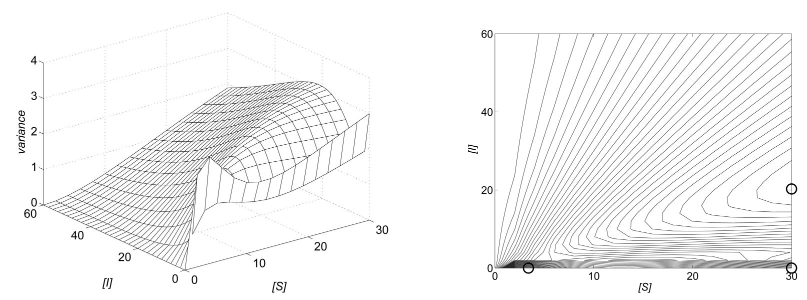


Figure 4: The maxima of variance function are at the optimum design support points, the maxima are equal to the number of the model parameters.

COST SAVINGS

THE OPTIMALLY DESIGNED EXPERIMENT

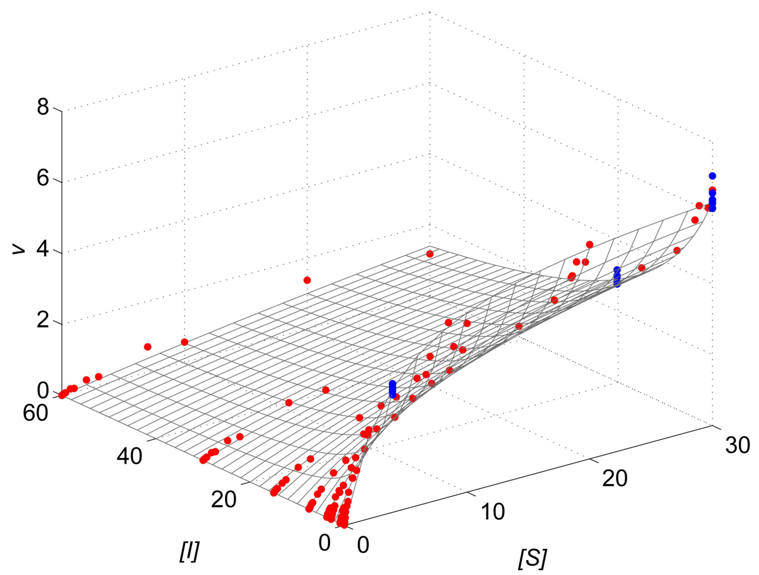


Figure 5: Competitive inhibition interaction between dextrometorphan, which is a substrate for the enzyme Cytochrome P450, 2D6, and an inhibitor of the enzyme, sertraline. The new observations (blue) and the old ones from the "rich" design (red) are shown at the picture, together with the model fitted to the old data.

The D-efficiency of the rich design compared to the optimum design is 18.21%; the theory says that approximately the same accuracy of parameter estimation can be obtained with 21 (instead of 120) observations now split equally between the three D-optimum support points, making 7 observations per point.

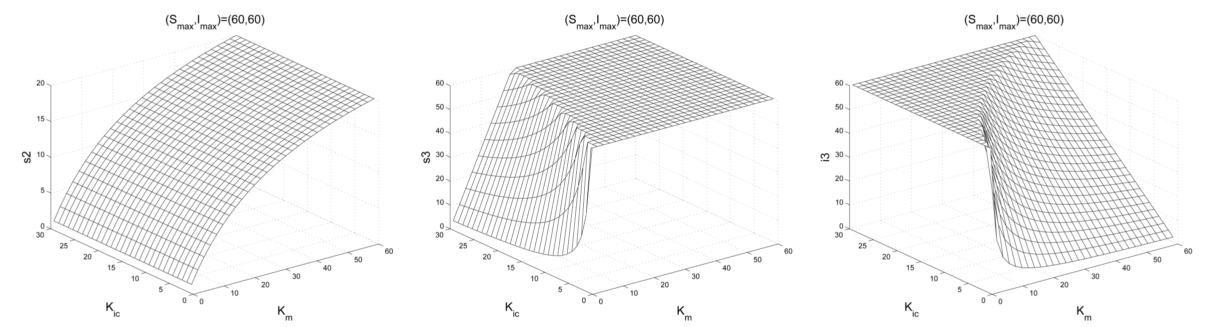
Table 1: Parameter estimates and associated standard errors from the "rich" data set and from observations coming from the D-optimum design.

	Rich data set ($n = 120$)		D-optimum ($n = 21$)	
Parameter	Estimate	Standard error	Estimate	Standard error
V	7.298	0.113	7.158	0.109
K_m	4.386	0.231	4.153	0.233
K_{ic}	2.582	0.144	2.089	0.127

DESIGN ROBUSTNESS

ADVANTAGES OF THE ANALYTICAL SOLUTIONS

- Optimum designs for any set of parameter values can be quickly calculated and their properties easily examined.

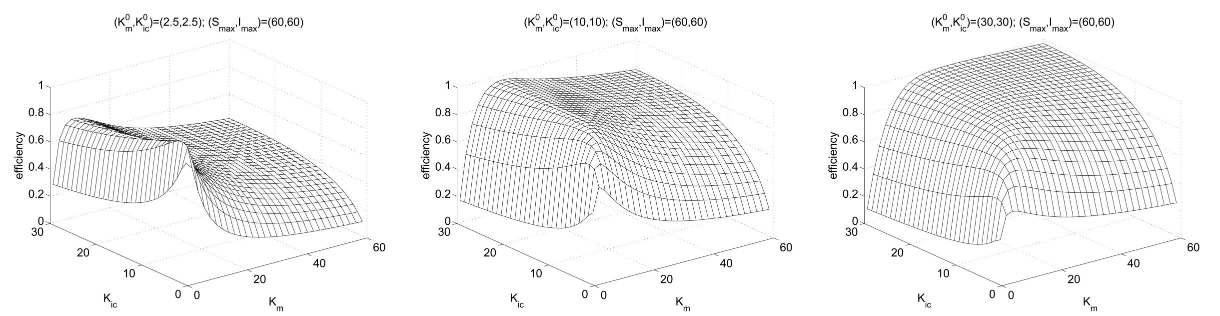


The "saturation" conditions for $[S]_3$ and for $[I]_3$ come from the analytical forms and they are

$$K_{ic} \geq \frac{K_m I_{\max}}{S_{\max} - K_m} \text{ for } [S]_3, \quad K_{ic} \geq \frac{I_{\max} - 2I_{\min}}{(S_{\max} + K_m)K_m} \text{ for } [I]_3.$$

- We can examine the properties of the designs' efficiency.

$$D_{\text{eff}}(\xi) = \left[\frac{\det\left(M(\xi, \boldsymbol{\vartheta}^0)\right)}{\det\left(M(\xi_D^\star, \boldsymbol{\vartheta}^0)\right)} \right]^{1/p}.$$



The implication for design is clear. Prior parameter values should be chosen for the locally optimum design that are toward the upper range of those thought plausible.

TWO COMPETING MODELS

NON-COMPETITIVE INHIBITION

- Non-competitive inhibitors have identical affinities for E and ES, that is $K_{ic} = K_{iu}$

The velocity equation is:

$$v = \frac{V[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_{ic}}\right)}$$

where K_{ic} is the inhibition constant.

MIXED INHIBITION

- Mixed-type inhibitors bind to both E and ES, but their affinities for these two forms of enzyme are different, that is $K_{ic} \neq K_{iu}$.

The velocity equation is:

$$v = \frac{V[S]}{K_m\left(1 + \frac{[I]}{K_{ic}}\right) + [S]\left(1 + \frac{[I]}{K_{iu}}\right)}$$

where K_{iu} is a dissociation constant.

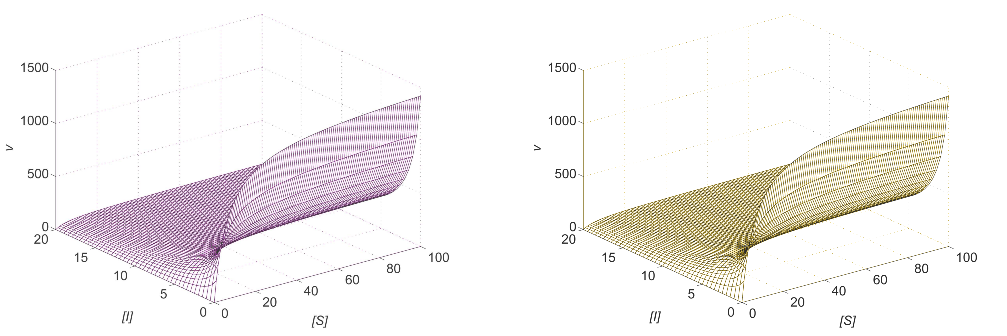


Figure 6: Two surfaces representing the enzyme kinetics: which is the "true" one? Non-competitive (left) or mixed (right)?

DESIGN PROBLEM

- Find optimum design for efficient discrimination between the models

We reparametrize the models so that the discrimination is achieved by testing equality of two parameters.

- Let $\theta_1 = 1/K_{ic}$ and $\theta_2 = 1/K_{iu}$. Then the mixed model becomes

$$v = \frac{V[S]}{K_m\,(1 + \theta_1[I]) + [S]\,(1 + \theta_2[I])}$$

- Reparameterize: $\theta_1 = \theta + \delta$ and $\theta_2 = \theta - \delta$, to obtain

$$v = \frac{V[S]}{(K_m + [S])\,(1 + \theta[I]) + \delta[I]\,(K_m - [S])} \qquad (\star)$$

- This model reduces to a non-competitive model when $\delta = 0$, equivalent to $K_{ic} = K_{iu}$.

T-optimality: High power for testing

$$H_0 : \delta = 0$$
$$H_1 : \delta = \delta^0 \quad \text{where } \delta^0 \neq 0$$

D_S-optimality: Precise estimation of δ

In linear models, when $s = 1$ (one parameter of interest) D_s and T-optimality criteria are equivalent, i.e., give the same designs. Do the designs differ when models are non-linear?

T-OPTIMUM DESIGNS

- Maximize power of the test that model 1 is true.
- The criterion function is:

$$\Delta(\xi, \psi_1^0) = \int_{\mathcal{X}} \left\{ \eta_1(x, \psi_1^0) - \eta_2(x, \widehat{\psi}_2(\xi)) \right\}^2 d\xi(x),$$

  where

$$\widehat{\psi}_2(\xi) = \arg\min_{\psi_2} \int_{\mathcal{X}} \left\{ \eta_1(x, \psi_1^0) - \eta_2(x, \psi_2) \right\}^2 d\xi(x).$$

- Here $\psi_1 = (V, K_m, \theta, \delta)$ and $\psi_2 = (V, K_m, \theta)$, that is η_1 is as in $(\star)$; η_2 as well, but with $\delta = 0$.

D$_S$-OPTIMUM DESIGNS

Model η_1 is linearized with respect to the parameters ψ_1. Vector ψ_1 and the information matrix are partitioned as follows

$$\psi_1 = (\psi, \delta), \quad \text{where } \psi = (V, K_m, \theta)$$

and

$$M(\xi) = \begin{pmatrix} M_{11}(\xi) & M_{12}(\xi) \\ M_{21}(\xi) & M_{22}(\xi) \end{pmatrix}$$

Then, the D$_S$-optimum design for δ maximizes the determinant:

$$|M_{22}(\xi) - M_{21}(\xi) M_{11}^{-1}(\xi) M_{21}^{\mathrm{T}}(\xi)| = |M(\xi)| / |M_{11}(\xi)|.$$

T-optimality does not involve any linearization. For nonlinear models D_1 and T-optimum designs are not in general identical. This arises because the D_1-optimality criterion is used for discriminating between models that are linearized with respect to ψ_1 at $(\psi^0, 0)$, while the T-optimality criterion is applied to the original model and depends on the nominal values (ψ^0, δ^0).

RESULTS (Atkinson and Bogacka, 2012)

Here we find

- T-optimum designs when the full non-linear model is true with parameters (ψ^0, δ^0)
- D$_S$-optimum designs when the prior is $(\psi^0, 0)$
- D$_S$(T)-optimum designs when the prior is $(\widehat{\psi}(\xi^\star), 0)$, where $\xi^\star$ denotes the T-optimum design.
- D-optimum design for ψ_1.

All designs have the form

$$\xi^\star = \begin{Bmatrix} ([S]_{\max}, [I]_{\min}) & ([S]_2, [I]_{\min}) & ([S]_{\max}, [I]_3) & ([S]_4, [I]_4) \\ w_1 & w_2 & w_3 & w_4 \end{Bmatrix}$$

- The D-optimum design for ψ_1 has all the weights equal and the support points have analytical solutions; they are functions of the parameters.
- Other designs have to be found numerically.
- The weights of T- and D$_S$(T)-optimum designs (but not D$_S$) follow the pattern of

$$w_1 = 0.5 - 2w_2, w_2 = w_3, w_4 = 0.5.$$

- The T-optimum design approaches the D_1-optimum design as $\delta^0 \to 0$, c.f, Lopez-Fidalgo et al.(2008).

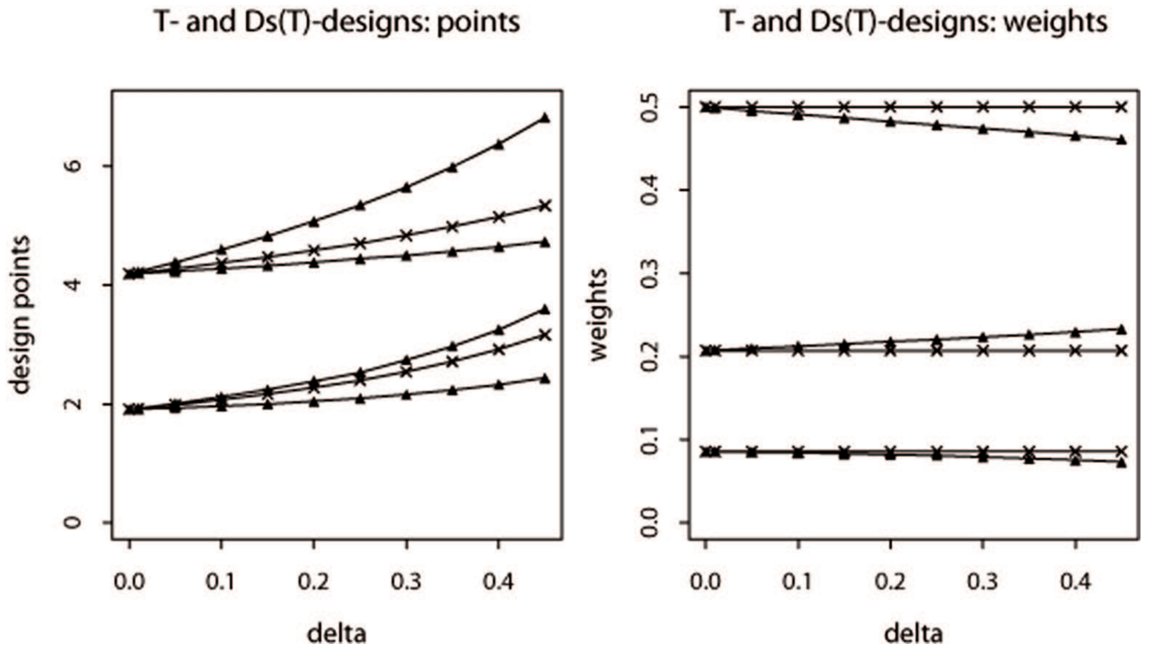


Figure 7: Enzyme kinetics: divergence of T- and D_s(T)-optimum designs as δ^0 increases: ▲, T-optimum designs; $\times$, D_s(T)-optimum designs. Left-hand panel, design points: upper triple, $[S]_4$ and $[S]_2$, equal for the D_s(T)-optimum designs; lower triple, $[I]_3$ and $[I]_4$, again equal for the D_s(T)-optimum designs. Right-hand panel, design weights: upper pair of curves, w_4; central pair, $w_2 = w_3$; bottom pair w_1.

EFFICIENCY

We define the T-efficiency of a design ξ as follows

$$T_{\text{eff}}(\xi) = \frac{\Delta(\xi)}{\Delta(\xi_T^\star)},$$

where $\xi_T^\star$ is the T-optimum design.

Design	T-efficiency $\delta = 0$	0.1	0.2	0.3	0.45
D	72.12	71.77	71.21	70.43	68.78
T	100	100	100	100	100
D_S	100	99.47	97.89	95.17	88.01
D_S(T)	100	99.79	99.15	98.00	95.02
Rich	1.11	1.38	1.73	2.22	3.41

Table 2: D_S(T)-optimum designs are more efficient than D_S when compared with T-optimum designs for non-linear models.

CONCLUSIONS

- Interestingly, all designs have the same border points.
- D_S(T)-optimum designs are more efficient than D_S when compared with T-optimum designs for non-linear models.
- Linearization decreases the efficiency of the design.
- How much does it depend on the parameter non-linearity of the model?
- Are there other values of the parameters to give even better efficiencies than those of D_S(T)?
- D_S- and D_S(T)-optimum design “tend” to T-optimum design when $\delta \to 0$.
- D_s-optimum designs are much easier to compute than T-optimum designs are.

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