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Monotonic Single-index Models to Assess Drug Interactions

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Abstract

Although single-index models have been extensively studied, the monotonicity of the link function f in the single-index model is rarely studied. In many situations, it is desirable that f is monotonic, which results in a monotonic single-index model that can be very useful in economics and biometrics. In this article, we propose a monotonic single-index model in which the link function is constructed using penalized I-splines along with constraints on coefficients to achieve monotonicity of the link function f . An algorithm to estimate the single-index parameters and the link function is developed, and the sandwich estimate of the variance of the index parameters is provided. We propose to apply this monotonic single-index model to estimate the dose-response surface and assess drug interactions while considering the variability of the observed data. An extensive simulation study was carried out to evaluate the performance of the proposed monotonic single-index model. A case study is provided to illustrate the application of the proposed model to estimate the dose-response surface and assess drug interactions. Both the simulation and case study show that the proposed monotonic single-index model works very well.

Keywords

Single-index model; Drug interaction; I-spline; Dose-response surface

1. Introduction

Single-index models have been extensively studied in the statistical literature [1–6]. A single-index model can be considered as an extension of a general or generalized linear model. Recall that a general linear model is defined as $Y = X^T \alpha + \epsilon$, where X is a vector of covariates, say $X = (X_0, X_1, \dots, X_p)^T$, and $\alpha = (\alpha_0, \alpha_1, \dots, \alpha_p)^T$ is an unknown parameter vector. A generalized linear model is defined as $E(Y) = h(X^T \alpha)$, where h is a known

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monotonic link function [7]. A single-index model generalizes the linear regression model by replacing $X^T\alpha$ with a nonparametric function of $X^T\alpha$, say $f(X^T\alpha)$. That is, $Y = f(X^T\alpha) + \epsilon$, where f is an unknown univariate link function to be estimated. In the literature, f is usually estimated by using either a kernel spline [1–5] or a penalized spline [6]. Single-index models have wide application in econometrics such as discrete choice analysis [2, 5] and the credit scoring method [4], as well as in biometrics such as modeling dose-response relationships [2, 6]. Note that both α and f in the single-index model are unknown and need to be estimated. To ensure identifiability, one may add constraints on α by either restricting $\alpha_0 = 1$ or restricting $\|\alpha\| = \sqrt{\alpha_0^2 + \alpha_1^2 + \dots + \alpha_p^2} = 1$ and $\alpha_0 > 0$. Constraints on α using $\|\alpha\| = 1$ and $\alpha_0 > 0$ have been widely used in the literature [1–6]. In contrast, a constraint on α using $\alpha_0 = 1$ has rarely been studied, but is perfectly suitable for assessing drug interactions, which is the main focus of this article. The asymptotic properties for α have been well established [1–3, 5, 6]. A single-index model reduces the dimensionality from multivariate predictors to a univariate index z (say $z = X^T\alpha$), while still capturing important features in high-dimensional data [6]. Any interaction among the covariates can also be included in the single index z . Therefore, the single-index model can be considered as an extension of the covariate interaction model.

Although monotonic splines have been studied and applied to modeling dose-response relationships [8–11], monotonicity in the single-index model has been rarely studied. In certain applications, the link function f in the single-index model is required to be monotonic. For example, in combination drug studies, the dose-response relationship is often described by a response surface model [12–14], where the marginal dose-response curve is often assumed to be monotonic. In this article, we propose to estimate the link function by using penalized splines with I-splines as its basis functions [10]. The monotonicity of function f is achieved by adding constraints to the coefficients of the I-spline basis functions [8–10]. The variances of the estimated index parameters and the function f are estimated using the technique developed by Yu and Ruppert [6].

As stated, one of the most important applications of the monotonic single-index model is to estimate a dose-response surface and assess drug interactions [12–17]. Without loss of generality, let us denote the response at the combination dose (d_1, d_2) as y . A relative potency, say ρ , is often used to describe how effective drug 2 is relative to drug 1; that is, the effect of drug 2 at dose level d_2 when applied alone is the same as that of drug 1 at dose level ρd_2 . If we assume that the dose-response curve for drug 1 is $y = f(d_1)$, then the dose-response curve for drug 2 can be described as $f(\rho d_2)$. If the two drugs do not have any interaction, i.e., the combination is additive, then the effect of the combination dose (d_1, d_2) can be described by $f(d_1 + \rho d_2)$. If the effect at (d_1, d_2) is more (or less) than the effect of drug 1 at dose level $d_1 + \rho d_2$, then the combination dose (d_1, d_2) is synergistic (or antagonistic) [13, 15]. Plummer and Short [17] used a model of the form

$f(d_1 + \rho d_2 + k \sqrt{d_1 d_2})$ to identify and quantify the departure from additivity, where $k > 0$ indicates synergy of the combination dose at (d_1, d_2) , and $k < 0$ indicates antagonism of the combination dose at (d_1, d_2) . Kong and Lee [14] extended the model of Plummer and Short [17] by replacing k with a quadratic function of (d_1, d_2) , say $g(d_1, d_2)$, so that the model has the flexibility to capture different patterns of drug interaction, i.e., some combinations of

dosage may be synergistic and some may be antagonistic [18]. The models proposed by Plummer and Short [17] and Kong and Lee [14] can be written in terms of $f(x^T\alpha)$. For example, one may set $x^T\alpha = d_1 + \rho d_2 + \kappa \sqrt{d_1 d_2}$ for the model by Plummer and Short [17] and $x^T\alpha = d_1 + \rho d_2 + g(d_1, d_2) \sqrt{d_1 d_2}$ for the model by Kong and Lee [14]. Note that in both approaches ([17] and [14]), the link function f is assumed to be a monotonic parametric model. The estimates of α can be biased when the parametric function f is misspecified. To avoid the problem caused by the misspecification of f , we relax the conditions on f to the minimal, such that f is monotonic and has continuous first and second derivatives. The relaxation of the link function f to an unknown monotonic function can be achieved by applying the proposed monotonic single-index model in this article, where the single index is specified as in Kong and Lee [14]. To our knowledge, this is the first time that the monotonic single-index model has been studied in modeling the dose-response surface and assessing drug interactions in terms of synergy, additivity, and antagonism.

The rest of this article is organized as follows. In Section 2, we propose a monotonic single-index model and develop the algorithm to estimate the monotonic link function f and the index parameter α in the single-index model. In Section 3, we investigate how we can apply the monotonic single-index model to assess the interactions of two drug dose combinations. In Section 4, we describe an extensive simulation study to examine the performance of the proposed model. In Section 5, we apply the proposed monotonic single-index method to examine the interactions of two drugs as given by Harbron [19]. The last section is devoted to a discussion and future research possibilities.

2. Monotonic single-index model

2.1. Monotonic single-index model and estimation

Let us denote the observed covariates as x_i and the response variable as y_i ($i = 1, \dots, n$), where x_i could be a collection of covariates and their interactions. Without loss of generality, let us denote $x_i = (x_{i0}, x_{i1}, \dots, x_{ip})^T$. The relationship between x_i and y_i can be described by a function of the form

$$y_i = f(x_i^T \alpha) + \varepsilon_i, (i=1, \dots, n). \quad (1)$$

Here, $\alpha = (\alpha_0, \alpha_1, \dots, \alpha_p)^T$, f is an unknown monotonic function, and ε_i ($i = 1, \dots, n$) are identically and independently distributed variables with mean 0 and variance σ^2 . To ensure identifiability, we restrict $\alpha_0 = 1$. As in the classical single-index model, let us denote $z_i = x_i^T \alpha$ ($i = 1, \dots, n$). Here, z_i is called a single index for x_i and $\alpha = (\alpha_0, \alpha_1, \dots, \alpha_p)^T$ are called the index parameters. Let us denote a knot sequence $\tau = \{\tau_j\}_{j=1}^K$, where $\min_{1 \leq i \leq n} \{z_i\} = L = \tau_1 < \tau_2 < \dots < \tau_K = U = \max_{1 \leq i \leq n} \{z_i\}$. The function f is defined on the domain $[L, U]$ over which f is approximated by a piecewise polynomial function in each interval $[\tau_l, \tau_{l+1}]$, $l = 1, \dots, K-1$. The estimate for the function f is required to join smoothly in the two adjacent intervals, say $[\tau_l, \tau_{l+1}]$ and $[\tau_{l+1}, \tau_{l+2}]$ ($l = 1, \dots, K-2$). The most commonly used estimates for f are cubic splines, where f is approximated by a cubic polynomial in each

interval, and the estimates are joined at each knot with continuous first and second derivatives. There are different basis functions for cubic splines, such as B-splines, M-splines, and I-splines [10]. Among them, I-splines are found to be the most convenient basis function for constructing monotonic curves [10].

Let us first assume that the knots are known and fixed and defer the procedure for knot selection to the end of this subsection. In general, an I-spline basis function of degree k is determined by $k + 1$ knots, say $\{\tau_j, \tau_{j+1}, \dots, \tau_{j+k}\}$, and an I-spline basis function can be expressed as the integral of an M-spline:

$$I_j^k(z) = \int_L^z M_j^k(u) du, \quad (2)$$

where $z \in [L, U]$. The M-splines can be obtained iteratively by the following iterative formula:

$$M_j^k(z) = \frac{k \left[(z - \tau_j) M_j^{k-1}(z) + (\tau_{j+k} - z) M_{j+1}^{k-1}(z) \right]}{(k-1)(\tau_{j+k} - \tau_j)}, \quad (3)$$

with $k > 1$, and

$$M_j^1(z) = \begin{cases} \frac{1}{\tau_{j+1} - \tau_j}, & \tau_j \leq z < \tau_{j+1}; \\ 0, & \text{otherwise.} \end{cases} \quad (4)$$

Because the M-spline basis function $M_j^k(z)$ is a piecewise nonnegative polynomial function of degree $k - 1$, the corresponding I-spline basis function $I_j^k(z)$ is therefore a piecewise monotone polynomial function of degree k [11]. We set $k = 3$ for cubic splines for the rest of this article. Note that the I-spline function $I_j^k(z)$ can also be put in the following more convenient form [11],

$$I_j^k(z) = \begin{cases} 0, & j > l; \\ \sum_{m=j}^l \frac{\tau_{m+k+1} - \tau_m}{k+1} M_m^{k+1}(z), & l - k + 1 \leq j \leq l; \\ 1, & j < l - k + 1, \end{cases} \quad (5)$$

for any $z \in [\tau_l, \tau_{l+1})$. The form in (5) avoids the integral in equation (2), and is used to calculate I-splines in this article. The monotonic function f is approximated by a linear combination of the I-splines in the following form,

$$f(z) = \beta_0 + \sum_{j=1}^{K-3} \beta_j I_j^3(z) = \mathcal{J}(z)^T \beta, \quad (6)$$

subject to $\beta_j = 0$ for $j = 1, \dots, K-3$. Here, $\mathcal{J}(z)^T = (1, I_1^3(z), I_2^3(z), \dots, I_{K-3}^3(z))$. Note that we add the constraint on the parameters β_j ($j = 1$) but not on β_0 , which enables us to obtain a monotonically increasing function with any starting threshold value. The parameters $\beta^T = (\beta_0, \beta_1, \dots, \beta_{K-3})$ in equation (6) are obtained by minimizing the following penalized residual sum of squares (PRSS),

$$\text{PRSS} = \frac{1}{n} \sum_{i=1}^n \{y_i - f(z_i)\}^2 + \lambda \int_L^U f''(u)^2 du. \quad (7)$$

With $I_j^3(z) = \int_L^z M_j^3(u) du$ and $f(z) = \beta_0 + \sum_{j=1}^{K-3} \beta_j I_j^3(z)$, we have $f'(z) = \sum_{j=1}^{K-3} \beta_j M_j^3(z)$, and

$f''(z) = \sum_{j=1}^{K-3} \beta_j (M_j^3(z))'$. Here, $f'(z)$ and $f''(z)$ denote the first and second derivatives of $f(z)$, respectively. It is straightforward to show the second term in (7),

$$\lambda \int_L^U f''(u)^2 du = \lambda \int_L^U \left\{ \sum_{j=1}^{K-3} \beta_j (M_j^3(u))' \right\}^2 du, \text{ which can be written in the form of } \lambda \beta^T D \beta.$$

Here, D is a matrix with the first row and first column as zeroes and the rest as a $(K-3) \times (K-3)$ pentadiagonal matrix with the (j, j') entry as $\int_L^U [M_j^3(u)]' [M_{j'}^3(u)]' du$. A detailed calculation for the matrix D is in the Appendix. Thus, given the index values

$z_i = x_i^T \alpha$ ($i = 1, \dots, n$) and knot sequence, for each fixed λ , one can obtain the estimate of f by minimizing the PRSS in equation (7). The selection of λ can be obtained by the generalized cross-validation (GCV) score, which has the following form,

$$\text{GCV}(\lambda) = \frac{\frac{1}{n} \sum_{i=1}^n \{y_i - \hat{\beta}_0 - \sum_{j=1}^{K-3} \hat{\beta}_j I_j^3(z_i)\}^2}{\left\{1 - \frac{1}{n} \text{tr}(S_\lambda)\right\}^2}, \quad (8)$$

where $\text{tr}(S_\lambda) = \text{tr}\{\mathcal{Q}(\mathcal{Q}^T \mathcal{Q} + n\lambda D)^{-1} \mathcal{Q}^T\}$, and $\mathcal{J} = (1, I_1^3(z_i), \dots, I_{K-3}^3(z_i))_{1 \leq i \leq n}$ is an $n \times (K-2)$ matrix with the i^{th} row calculated at the i^{th} index value $z_i = x_i^T \alpha$ ($i = 1, \dots, n$). In our simulation and case study, we set a grid of 100 values for λ in $[10^{-10}, 10^{10}]$ by equally spacing λ in log-scale. Note that the tuning parameter λ controls the tradeoff between the goodness-of-fit and the smoothness of the estimated function f . The value of λ is selected as the one that minimizes the GCV scores. The optimal estimate of f for a given α is obtained by minimizing PRSS with the selected λ .

For a given f , one may obtain the estimate for α by minimizing the following residual sum of squares for errors (RSSE),

$$\text{RSSE}(\alpha) = \frac{1}{n} \sum_{i=1}^n \{y_i - f(x_i^T \alpha)\}^2, \quad (9)$$

where $x_i = (x_{i0}, x_{i1}, \dots, x_{ip})^T$ and $\alpha = (\alpha_0, \alpha_1, \dots, \alpha_p)^T$. To ensure identifiability, the first component of the index parameter α is restricted to 1, that is, $\alpha_0 = 1$. Let us denote $\alpha^T = (1, \tilde{\alpha}^T)$, where $\tilde{\alpha}^T = (\alpha_1, \dots, \alpha_p)$; and $x_i^T = (x_{i0}, \tilde{x}_i^T)$, where $\tilde{x}_i^T = (x_{i1}, \dots, x_{ip})$. In general, the minimizer for the RSSE in (9) can be obtained by solving the following estimating equation,

$$\frac{1}{n} \sum_{i=1}^n (y_i - f(x_i^T \alpha)) \frac{\partial f(x_i^T \alpha)}{\partial \tilde{\alpha}} = 0. \quad (10)$$

Consider the first order Taylor expansion for $f(x_i^T \alpha)$ at $x_i^T \alpha^{(0)}$,

$$f(x_i^T \alpha) \approx f(x_i^T \alpha^{(0)}) + f'(x_i^T \alpha^{(0)}) \tilde{x}_i^T (\tilde{\alpha} - \tilde{\alpha}^{(0)}) = f(x_i^T \alpha^{(0)}) - f'(x_i^T \alpha^{(0)}) \tilde{x}_i^T \tilde{\alpha}^{(0)} + f'(x_i^T \alpha^{(0)}) \tilde{x}_i^T \tilde{\alpha}, \quad (11)$$

where $\alpha^{(0)}$ is the known initial value for α . One can update $\tilde{\alpha}$ by solving the estimating equation

$$\frac{1}{n} \sum_{i=1}^n (y_i^* - \tilde{x}_i^{*T} \tilde{\alpha}) \tilde{x}_i^* = 0, \quad (12)$$

where $y_i^* = y_i - f(x_i^T \alpha^{(0)}) + f'(x_i^T \alpha^{(0)}) \tilde{x}_i^T \tilde{\alpha}^{(0)}$ and $\tilde{x}_i^* = f'(x_i^T \alpha^{(0)}) \tilde{x}_i$. The solution for the estimating equation (12) is equivalent to minimizing $\frac{1}{n} \sum_{i=1}^n (y_i^* - \tilde{x}_i^{*T} \tilde{\alpha})^2$, which can be implemented by a linear regression model. One can replace $\tilde{\alpha}^{(0)}$ with the currently available $\tilde{\alpha}$ and continue with the same procedure to obtain a better estimate of $\tilde{\alpha}$. This procedure may be repeated a number of times to obtain the optimal estimate of α for a given f . The final estimates for f and α can be obtained by the following iterative algorithm:

Step 0: Obtain an initial estimate for α . For example, take $\alpha^{(0)}$ as the least squares estimate for $y_i = x_i^T \alpha + \varepsilon_i (i=1, \dots, n)$, and set $\hat{\alpha} = \hat{\alpha}^{(0)} / \hat{\alpha}_0^{(0)}$.

Step 1: Estimate the link function f by carrying out the following steps:

- a. Form a knot sequence, say $\{\tau_1, \dots, \tau_K\}$, based on the single-index values $\hat{z}_i = x_i^T \hat{\alpha} (i=1, \dots, n)$ (see Remark 1 on knot selection at the end of this paragraph).
- b. For each fixed value of λ among the 100 values on a grid, we obtain the estimates of β (thus f) by minimizing the PRSS in equation (7), and calculate the GCV(λ). We choose λ as the one that minimizes the GCV score.
- c. Estimate f with the selected λ .

Step 2: Update the estimate of α by minimizing the RSSE in equation (9), where f is obtained in Step 1(c).

Step 3: Repeat step 1 and step 2 until α converges.

Remark 1 on knot selection—Note that the spline approximation is an ill-conditioned inverse problem and requires careful allocation of knots and thorough selection of the tuning parameter. For classical smoothing splines, the knots are selected as all unique values of z , and the tuning parameter is selected by the GCV method [20]. For penalized splines, the knots are usually selected as a smaller set from the unique index values. For example, the knots are placed at the quantiles of the observed index values [21], or placed equidistance on the space of the observed index values [22]. Yu and Ruppert [6] recommended using quantiles of the index values as the knots, where roughly 10 knots are selected. However, we found that the approach based on quantiles may select a large number of knots in a small area in which many observations are found, and select a few or no knots in the area in which the observations are small in number but are influential. Instead of selecting knots based on quantiles, we propose to select knots as a subset of distinct index values with the distance between any two knots being larger than a certain pre-specified value, say w_0 . That is, the index values are first set in order, say $z_{(1)} < z_{(2)} < \dots < z_{(n)}$, then $z_{(1)}$ is selected as the first knot (i.e., $\tau_1 = z_{(1)}$). The second knot τ_2 is selected as the index value that is closest to τ_1 under the restriction that $\tau_2 - \tau_1 \geq w_0$. The third knot is selected as the index value that is closest to τ_2 under the restriction that $\tau_3 - \tau_2 \geq w_0$. This procedure is continued until all index values are examined. In our simulation, we set $w_0 = 0.5$ so that roughly 10 knots were selected. This approach selects the representative index values to the knot sequence, and avoids selecting too many knots from a small area where many observations are found.

Remark 2 on estimating f in Step 1—We noticed that after a certain number of iterations, the selected values for tuning parameter λ ; in Step 1(b) are very close to each other. To reduce the computational burden, after a certain number of iterations, the tuning parameter can be fixed. Thus, β (i.e., f) in Step 2 only needs to be estimated once because λ is given.

2.2. Variance estimate for $\hat{\alpha}$ and $\hat{f}$

The asymptotic properties for $\hat{\alpha}$ in the single-index model have been studied by Härdle et al. [23] and Xia and Härdle [4], in which a kernel spline was used to estimate f . Yu and Ruppert [6] used penalized splines to estimate f . Our approach uses the framework of penalized splines, and the asymptotic normality for the estimates of $\hat{\alpha}$ and $\hat{\beta}$ holds [6]. Let us denote the parameters $\theta^T = (\tilde{\alpha}^T, \beta^T)$. We estimate θ by solving the following estimating equation,

$$\begin{pmatrix} \frac{1}{n} \sum_{i=1}^n (y_i - f(z_i)) \frac{\partial f(z_i)}{\partial \alpha} \\ \frac{1}{n} \sum_{i=1}^n \left[-(y_i - f(z_i)) \frac{\partial f(z_i)}{\partial \beta} + \lambda D\beta \right] \end{pmatrix} = 0, \quad (13)$$

where $z_i = x_i^T \alpha = x_{i0} + \tilde{x}_i^T \tilde{\alpha}$, $f(z_i) = \beta_0 + \sum_{j=1}^{K-3} I_j^3(z_i) \beta_j$, and D is a block-diagonal matrix with the first diagonal entry being zero and the second being the pentadiagonal matrix as specified in the Appendix. Let

$$\mathcal{J}(z_i) = \left(1, I_1^3(z_i), I_2^3(z_i), \dots, I_{K-3}^3(z_i) \right)^T, \quad (14)$$

and denote

$$\psi_i(\theta; \lambda) = \begin{pmatrix} -(y_i - f(z_i)) f'(z_i) \tilde{x}_i \\ -(y_i - f(z_i)) \mathcal{J}(z_i) + \lambda D \beta \end{pmatrix}. \quad (15)$$

Let $\theta(\lambda)$ solve $\frac{1}{n} \sum_{i=1}^n E \psi_i\{\theta(\lambda); \lambda\} = 0$, and $\hat{\theta}(\lambda)$ solve $\frac{1}{n} \sum_{i=1}^n \psi_i(\theta; \lambda) = 0$, then one can show that $\hat{\theta}(\lambda)$ is consistent for $\theta(\lambda)$ [4, 23], and that

$$n^{1/2}(\hat{\theta}(\lambda) - \theta(\lambda)) \xrightarrow{D} N\left(0, H^{-1}(\theta(\lambda)) G(\theta(\lambda)) H^{-T}(\theta(\lambda))\right),$$

where

$$H(\theta) = \frac{1}{n} \sum_{i=1}^n \frac{\partial \psi_i}{\partial \theta^T} = \frac{1}{n} \sum_{i=1}^n \begin{bmatrix} f'(z_i)^2 \tilde{x}_i \tilde{x}_i^T & f'(z_i) \tilde{x}_i \mathcal{J}(z_i)^T \\ f'(z_i) \mathcal{J}(z_i) \tilde{x}_i^T & \mathcal{J}(z_i) \mathcal{J}(z_i)^T + \lambda D \end{bmatrix},$$

$$G(\theta) = \frac{1}{n} \sum_{i=1}^n \psi_i(\theta) \psi_i(\theta)^T. \quad (16)$$

These asymptotics justify the sandwich estimator of the covariance matrix of $\theta(\lambda)$:

$$V_{sw}(\hat{\theta}(\lambda)) = \frac{1}{n} H^{-1}(\hat{\theta}(\lambda)) G(\hat{\theta}(\lambda)) H^{-T}(\hat{\theta}(\lambda)). \quad (17)$$

3. Application of monotonic single-index model to assess drug interactions

The monotonic single-index model developed here can be applied to assess drug interactions between two or more drugs. Without loss of generality, let y_i denote the response observed at the combination dose level (d_{1i}, d_{2i}) ($i = 1, \dots, n$). In the case in which drug 1 is applied alone, d_{2i} is set to zero. Similarly, d_{1i} is set to zero if drug 2 is applied alone. Plummer and Short [17] proposed a model of the form $f_d(d_1 + \rho d_2 + \kappa \sqrt{d_1 d_2})$ to identify drug interactions using the parameter κ . Kong and Lee [14] extended Plummer and Short's model [17] to the following form $y = f_d(d_1 + \rho d_2 + g(d_1, d_2; \kappa) \sqrt{d_1 d_2}) + \varepsilon$. Here, a quadratic function of (d_1, d_2)

in the form of $g(d_1, d_2; \kappa) = \kappa_0 + \kappa_1 \sqrt{d_1} + \kappa_2 \sqrt{d_2} + \kappa_3 d_1 + \kappa_4 d_2 + \kappa_5 \sqrt{d_1 d_2}$ is used to capture different patterns of drug interactions. In these approaches, the function f is assumed to be a known parametric function. Misspecification of f may lead to biased estimates of the parameter κ in the models by Plummer and Short [17] and Kong and Lee [14]. The proposed monotonic single-index model provides an ideal approach to assess drug interactions by relaxing the assumption of the link function f to the minimal: f is monotonic and has first and second derivatives. That is, the model has the following form,

$$y_i = f(d_{1i} + \rho d_{2i} + g(d_{1i}, d_{2i}; \kappa) \sqrt{d_{1i} d_{2i}}) + \varepsilon_i = f(x_i^T \alpha) + \varepsilon_i (i=1, \dots, n), \quad (18)$$

subject to $\alpha_0 = 1$ and f being monotonic. Here,

$$\alpha^T = (1, \rho, \kappa_0, \kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5) = (\alpha_0, \alpha_1, \dots, \alpha_7) = (1, \tilde{\alpha}^T), \quad (19)$$

and

$$x_i^T = \left(d_{1i}, d_{2i}, \sqrt{d_{1i} d_{2i}}, d_{1i} \sqrt{d_{2i}}, \sqrt{d_{1i} d_{2i}}, \sqrt{d_{1i}^3 d_{2i}}, \sqrt{d_{1i} d_{2i}^3}, d_{1i} d_{2i} \right). \quad (20)$$

Recall that we assess whether a combination dose (d_1, d_2) is synergistic by examining whether the function $g(d_1, d_2; \kappa)$ is significantly greater than zero. Therefore, it is important to estimate the quantity of the function $g(d_1, d_2; \kappa)$ and its variation so that the inference for drug interactions can be made with statistical rigor. Note that the function $g(d_1, d_2; \kappa)$ can be written as a linear function of $\tilde{\alpha}$, say $g(d_1, d_2; \kappa) = u^T \tilde{\alpha}$, where

$u^T = (0, 1, \sqrt{d_1}, \sqrt{d_2}, d_1, d_2, \sqrt{d_1 d_2})$. From Section 2, we can obtain the estimate for $\tilde{\alpha}$, say $\hat{\tilde{\alpha}}$, and the variance for $\hat{\tilde{\alpha}}$, say $\text{Var}(\hat{\tilde{\alpha}})$. Thus, $g(d_1, d_2; \kappa)$ can be estimated by $u^T \hat{\tilde{\alpha}}$, and its variance can be estimated by $u^T \text{Var}(\hat{\tilde{\alpha}}) u$. The $100(1 - \alpha)\%$ confidence interval (CI) for

$g(d_1, d_2; \kappa)$ can be constructed as $u^T \hat{\tilde{\alpha}} \pm z_{\alpha/2} \sqrt{u^T \text{Var}(\hat{\tilde{\alpha}}) u}$, where $z_{\alpha/2}$ is the upper $\alpha/2$ quantile of the standard normal distribution. For each combination dose (d_1, d_2) , one can assess whether $g(d_1, d_2; \kappa)$ is significantly different from zero. If the estimate for $g(d_1, d_2; \kappa)$ and its 95% confidence interval (CI) indicate that $g(d_1, d_2; \kappa)$ is significantly greater than zero, then we conclude that the combination dose (d_1, d_2) is synergistic. If the estimate for $g(d_1, d_2; \kappa)$ and its 95% CI indicate that $g(d_1, d_2; \kappa)$ is significantly less than zero, then we conclude that the combination dose (d_1, d_2) is antagonistic. Otherwise, we conclude that the combination dose (d_1, d_2) is additive. Thus, different patterns of drug interactions within the same experiment can be captured.

In addition, we can obtain the response profile for each combination dose by plugging $\hat{\tilde{\alpha}}$ and $\hat{\beta}$ into equation (6):

$$\hat{f}_d(d_1, d_2) = \hat{\beta}_0 + \sum_{j=1}^{K-3} \hat{\beta}_j I_j^3(x(d_1, d_2)^T \hat{\alpha}), \quad (21)$$

where

$$x(d_1, d_2)^T = (d_1, d_2, \sqrt{d_1 d_2}, d_1 \sqrt{d_2}, d_2 \sqrt{d_1}, \sqrt{d_1^3 d_2}, \sqrt{d_1 d_2^3}, d_1 d_2) = (d_1, \tilde{x}(d_1, d_2)^T), \quad (22)$$

and $\hat{\alpha}^T = (1, \hat{\alpha}^T)$. The variance for $f_d(d_1, d_2)$ can be obtained by using the delta method [24]:

$$\text{Var}(\hat{f}_d(d_1, d_2)) = \left(\frac{\partial \hat{f}_d}{\partial \hat{\alpha}^T}, \frac{\partial \hat{f}_d}{\partial \hat{\beta}^T} \right) \text{Var} \begin{pmatrix} \hat{\alpha} \\ \hat{\beta} \end{pmatrix} \begin{pmatrix} \frac{\partial \hat{f}_d}{\partial \hat{\alpha}} \\ \frac{\partial \hat{f}_d}{\partial \hat{\beta}} \end{pmatrix}, \quad (23)$$

where

$$\frac{\partial \hat{f}_d}{\partial \hat{\alpha}} = \sum_{j=1}^{K-3} \hat{\beta}_j M_j^3(x(d_1, d_2)^T \hat{\alpha}) \tilde{x}(d_1, d_2), \quad (24)$$

and

$$\frac{\partial \hat{f}_d}{\partial \hat{\beta}} = \left(1, I_1^3(x(d_1, d_2)^T \hat{\alpha}), \dots, I_{K-3}^3(x(d_1, d_2)^T \hat{\alpha}) \right)^T. \quad (25)$$

Thus, one can estimate the dose-response surface as well as its 95% point-wise confidence surfaces and gauge the overall response profile. In the following section, we describe the results of a simulation study to examine the performance of the proposed model. In section 5, we provide a case study to illustrate how to use this method to assess drug interactions and gauge the dose-response profile.

4. Simulation

We carried out a simulation study to examine the following aspects of the performance of the proposed model: (i) the accuracy of the proposed single-index model in estimating the single-index parameters α ; (ii) a comparison of the estimated parameter α based on the single-index models with those obtained from parametric models, when specified correctly as well as misspecified; (iii) the performance of the proposed single-index model in assessing drug interactions and estimating the dose-response surface; and (iv) the small-sample properties of the delta method in estimating the variances for the response surface function f and the interaction function g . In the drug combination study, the Emax model

[25] of the form $E = \frac{E_{\max}d}{ED_{50} + d}$ is often used to describe the dose-response curve when a single drug is applied. Here, d is the dose of a drug, $E_{\max}$ is the maximum effect of a drug, and ED_{50} is the median effective dose of a drug that produces 50% of the maximum effect. To examine the performance of the proposed model, let us assume that drug 1 and drug 2 are equally potent, and both drugs have a 100% maximum effect with a median effective dose of 1. That is, the dose-response curves for drug 1 and drug 2 follow the equation in [14]

$$E = \frac{100d}{1+d}, \quad (26)$$

where the response increases as the dose increases. Because drug 1 and drug 2 are equally potent, the relative potency $\rho = 1$. Suppose that the response at combination dose (d_1, d_2) is captured by

$$Y = \frac{100(d_1 + d_2 + g(d_1, d_2; \kappa) \sqrt{d_1 d_2})}{1 + (d_1 + d_2 + g(d_1, d_2; \kappa) \sqrt{d_1 d_2})} + \varepsilon, \quad (27)$$

where $g(d_1, d_2; \kappa) = 0.9 - 0.3d_1 - 0.3d_2$. Then, the underlying dose response surface can be described as

$$Y = \frac{100x^T \alpha}{1 + x^T \alpha} + \varepsilon, \quad (28)$$

where $x^T = (d_1, d_2, \sqrt{d_1 d_2}, d_1 \sqrt{d_2}, d_2 \sqrt{d_1}, \sqrt{d_1^3 d_2}, \sqrt{d_1 d_2^3}, d_1 d_2)$, $\alpha^T = (1, 1, 0.9, 0, 0, -0.3, -0.3, 0)$, and $\varepsilon \sim N(0, \sigma^2)$. We set the dose levels for drug 1 and drug 2 as (0, 0.1, 0.5, 1, 2, 4), and combined each dose level of drug 1 with each dose level of drug 2. We set the replicates for each sample as 3. Thus, the total sample size was $3 \times 6 \times 6 = 108$. We generated each response at the combination dose (d_1, d_2) from the underlying model (28) with $\sigma = 1$ and 0.1, respectively. We fitted each data set with the proposed monotonic single-index model, the true underlying model $E = (100x^T \alpha)/(1 + x^T \alpha) + \varepsilon$, and the misspecified model $E = x^T \alpha + \varepsilon$. We obtained the estimated parameters and the corresponding standard errors of α under each model. We repeated this process 1000 times. In Table 1, we report the average of the estimated parameters (Est), the empirical standard deviation calculated from the 1000 estimates for α (SE.emp), and the average of the 1000 model-based standard errors (SE) of α for each model. Based on Table 1, we conclude that (i) both the proposed single-index model and the underlying true model provide unbiased estimates for α ; (ii) when the model is misspecified, the estimates for α can be seriously biased; (iii) the empirical standard deviation (see “SE.emp” under the column “Single-index model” in Table 1) is close to the average of the standard error based on the sandwich estimator proposed in Section 2.2, indicating that the proposed sandwich estimate captures the variability of the estimated index parameter reasonably well; and (iv) the variances for index parameters α obtained from the single-index model are not far from those resulting from the underlying

model, indicating that the single-index model provided estimates of α that were almost as efficient as those obtained from the underlying true model.

To examine the performance of the proposed monotonic single-index model in estimating the dose-response surface function f and the interaction function g , as well as to examine the small-sample properties of the delta method in estimating their variances, we generated 1000 simulated data from the underlying model (27) and estimated the functions f and g and their variances at each combination dose for the simulated data. The average of the 1000 estimates (Est.) of functions f and g at each combination dose, the empirical standard deviation (SE.emp) based on the 1000 estimates, and the average of the 1000 delta-method-based standard errors are summarized in Table 2. Based on Table 2, we conclude that (i) the estimates for both f and g are close to the underlying values; (ii) the empirical standard deviation is close to the average of the delta-method-based standard error, indicating that the delta method performs well; and (iii) the standard error decreases as σ decreases, as expected. Figure 1 illustrates the performance of the proposed method in estimating function f and g and their variances. From Figure 1(A1 and A2), we see that the patterns of the underlying response surface and the fitted response surface are very similar. From Figure 1(A3–A6), we see that the fitted lines are very close to the underlying line in each panel, and the 95% confidence bounds cover the underlying curve. From Figure 1(B2), we see that the g function in the shaded area is not significantly different from zero, which indicates that the combination doses in this area are additive. Comparing the 10 fitted curves in each one of the panels A3–A6 and B3–B6 in Figure 1, it seems that the fitted dose-response curves in panels A3–A6 have less variability than the fitted g functions in panels B3–B6. The function g is a polynomial function, which is a component of the index value. That is,

$x^T \alpha = d_1 + \rho d_2 + g(d_1, d_2; \kappa) \sqrt{d_1 d_2}$. The index parameters (i.e., α) and the link function (i.e., f) are estimated by minimizing the RSSE and the PRSS, respectively. The RSSE measures the goodness-of-fit of the response variable. The fact that α is estimated by minimizing the RSSE may explain why the variability of the estimated response surface function f was smaller than that of the estimated interaction function g . However, from the results in Figure 1, we can see that the proposed single-index model estimates the underlying response surface and the function g very well, and all 10 fitted curves are very close to each underlying curve.

To examine the performance of the proposed method under different designs such as the ray design [13], we carried out the simulation study with data generated from the two marginal dose-response curves along with the dose-response over the three rays: the first ray has the combination dose (d_1, d_2) with $d_1/d_2 = 1$ and the observed responses at the combination doses (0.1, 0.1), (0.5, 0.5), (1, 1), (2, 2), and (4, 4); the second ray has the combination dose (d_1, d_2) with $d_1/d_2 = 1/2$ and the observed responses at the combination doses (0.1, 0.2), (0.5, 1), (1, 2), and (2, 4); and the third ray has the combination dose (d_1, d_2) with $d_1/d_2 = 2/1$ and the observed responses at the combination doses (0.2, 0.1), (1, 0.5), (2, 1), and (4, 2). Thus, we used 13 instead of 25 combination doses for this simulation study. The mean of the 1000 estimates, the empirical standard error, and the mean of the model-based standard errors for the parameter α are reported in Table S1, and the simulation results for the response surface function f and the interaction function g are reported in Table S2 in the

Supplementary Material. Based on the simulation results, we conclude that the proposed method can be applied to data obtained through different experimental designs, such as the factorial design and ray design [13]. In the following section, we apply the proposed single-index model to assess two drug interactions from an *in vitro* drug combination study by Harbron [19].

5. Case study

A flexible unified approach to the analysis of preclinical drug combination studies was developed by Harbron [19], where a data set from an *in vitro* drug combination experiment was studied as an example case. In the study, two compounds (drug A and drug B) were dosed as monotherapy along 9 dose levels: 0.037, 0.11, 0.33, 1, 3, 9, 27, 81, and 243 [19]. The two compounds were combined in a factorial design for all of the 6 lower dose levels from 0.037 to 9. All design points had three readings for the percentage of growth inhibition within a cell culture. The percentage of growth inhibition was calculated from the optical density, corrected for background and normalized against an average of no treatment response [19].

We applied the proposed single-index model to the case study data. To start, we set a grid of size 100 for the tuning parameter λ in the interval $[10^{-10}, 10^{10}]$ by equally spacing $\log \lambda$ on $[-10, 10]$. We followed the algorithm in Section 2 to obtain the optimal λ and knot sequence. The final tuning parameter λ selected was 0.077, and 14 knots were selected with $w_0 = 2$. The final estimated $\tilde{\alpha}$ were (0.363, -0.635, 1.717, 0.547, 0.270, -0.050, -0.674) with standard errors (0.033, 0.822, 1.081, 0.553, 0.376, 0.137, 0.210).

To examine the goodness-of-fit of the fitted response surface, we plotted the contour of the observed growth inhibition on the lower 6×6 factorial design points (Figure 2(A1)), as well as the contour of the fitted response surface at the same combination doses (Figure 2(A2)). In addition, we plotted the observed response (% inhibition) versus the indices (circles), and the fitted responses versus the indices (solid line) in Figure 2(B1). We also plotted the observed values versus the dose of drug A (circles) and the fitted function $\hat{f}$ versus the dose of drug A (lines) when only drug A was applied (Figure 2(B2)), and when equal amounts of drug A and drug B were combined (Figure 2(B3)). The plot of the observed values and the fitted function $\hat{f}$ versus drug B (not shown) is similar to that for drug A in Figure 2(B2). To facilitate viewing, the single index in Figure 2(B1) and the dose levels in Figure 2(B2 and B3) are shown on a log-transformed scale, although the analysis was performed on the original dose scale. From Figure 2(A1 and A2), we see that the contour plot of the fitted dose-response surfaces is very similar to the contour plot of the observed data. From Figure 2(B1–B3), we observe that the model fits the data very well for all data (Figure 2(B1)), marginal dose-response (Figure 2(B2)), and typical combinations (Figure 2(B3)).

To examine the interactions of drug A and drug B, we made a contour plot of the polynomial function $g(d_1, d_2; \kappa)$ (see solid lines in Figure 2(C)). We constructed 95% point-wise confidence surfaces for the function $g(d_1, d_2; \kappa)$, and plotted the contour lines of level zero of the confidence surfaces as dotted lines (Figure 2(C)). Indeed, in this case study, the upper 95% confidence surface is always greater than zero, and the dotted lines in the middle in

Figure 2(C) are the zero-contour lines of the lower 95% confidence surface. Therefore, the function g in the shaded region is not significantly different from zero, indicating that the combination doses in these regions are additive. The function g in the unshaded region is significantly greater than zero, indicating that the combination doses in this region are synergistic. We compared our findings with those from Harbron [19]. The majority of the findings on the assessment of drug interactions are the same except at the three combination doses (0.111, 0.111), (1, 0.111), and (9, 9). Our method uses all data simultaneously to fit the single-index model, provides smooth boundaries and regions for different patterns of drug interactions, and leads to more robust inferences for drug interactions.

6. Discussion and future research

We have developed a monotonic single-index model and applied it to assess the interaction of two drugs when used in combination. Although the theoretical frameworks for spline smoothing and single-index models have been well developed, monotonicity in the single-index model has rarely been studied. In this article, we used I-spline functions along with constraints on the coefficients to achieve monotonicity of the link function, thus making it suitable for assessing drug interactions. The monotonic single-index model in the current article is developed under the framework of regression splines. Traditionally, knots are selected as the quantiles of observed indices. Here, we selected the knots according to the relative locations of the single indices, allowing us to avoid selecting too many knots from a small area where many observations are found. The criteria for assessing drug interactions have been established on the basis of the Loewe additivity model [12–17], which states that two drugs do not interact if the effect of the two drugs at dose (d_1, d_2) is the same as the effect of drug 1 alone at dose level $d_1 + \rho d_2$, where ρ is the relative potency, indicating that the effect of drug 2 at dose level d_2 is the same as the effect of drug 1 at dose level ρd_2 based on the individual dose-response profiles when each drug is applied alone. In the form of the index $d_1 + \rho d_2 + g(d_1, d_2; \kappa) \sqrt{d_1 d_2}$, the third term is suitable to capture different patterns of drug interactions. Thus, we have creatively applied single-index models to assess preclinical drug interactions. Based on the simulation and case study, the proposed monotonic single-index modeling approach works well in assessing drug interactions. The proposed model estimates the index parameters using all observed data and thus tends to be more robust for assessing drug interactions compared to using a two-stage semiparametric model [26] or using only marginal data and certain combination data [19]. Although our findings in assessing drug interactions are largely consistent with those of Harbron [19], our approach provides smooth boundaries and regions for different patterns of drug interactions and tends to be more robust in estimating drug interactions.

In the simulation study, we first examined the performance of the proposed method when data were generated from a factorial design with a setting similar to that in [26], and then examined the performance of the proposed method when data were generated from a ray design. The proposed method performed well under both designs. Theoretically, the proposed method could be applied to experimental data generated from any type of design such as a factorial design, ray design [13], uniform design [27], or D-optimal design [28]. The D-optimal design [28] requires minimizing the determinant of the covariance matrix of

the parameters. When the link function is known, optimal designs, including the D-optimal design, have been investigated by Stufken and Yang [29]. The D-optimal design may contain only the dimension of α support points [28, 29]. When the link function is unknown, Agarwal and Studden [30] reported an asymptotic design and estimation using linear splines. It may be challenging to use the optimal design for a single-index model. An alternative approach is to obtain the optimal design first by assuming that the link function is known, and then to add a few extra support points when the link function is unknown and needs to be estimated. For *in vivo* experiments or clinical studies, for which the admissible number of support points is usually very limited, the method that requires fewer support points is preferred. Using the optimal design for a single-index model may deserve further study. The proposed monotonic single-index model may be incorporated with the optimal design to provide an effective way for assessing drug interactions.

The proposed monotonic single-index model requires estimating both the index parameter α and the link function f ; whereas the model by Plummer and Short [17] and its extension by Kong and Lee [14] require estimating only the index parameter because the link function is known. By the parsimonious principle, if the goodness-of-fit of a simple model is adequate, the simple model is preferred. The goodness-of-fit for Plummer and Short's model [17] or its extension by Kong and Lee [14] could be examined by test statistics based on the empirical process of the covariates marked by the residuals [31, 32]. A goodness-of-fit test with a similar idea for the single-index model has been developed [33]. In application, one may first examine whether the goodness-of-fit of the parametric model is adequate before applying the monotonic single-index model. If the goodness-of-fit of the parametric model is not adequate, then one may consider applying the single-index model. Furthermore, one may check the goodness-of-fit of the single-index model. Inadequacy of the goodness-of-fit of a single-index model may lead to the decision to use a more complex model for assessing drug interactions, such as the two-stage semiparametric model [26]. Goodness-of-fit tests specific to models for assessing drug interactions deserve further investigation, which is beyond the scope of this article.

The proposed monotonic single-index model can be extended to assess the interactions of multiple drugs by extending the single-index expression. For example, a possible extension is to set the index values as

$$x^T \alpha = d_1 + \alpha_1 d_2 + \alpha_2 d_3 + g_{12}(d_1, d_2; \kappa_1) \sqrt{d_1 d_2} + g_{13}(d_1, d_3; \kappa_2) \sqrt{d_1 d_3} + g_{23}(d_2, d_3; \kappa_3) \sqrt{d_2 d_3} + g_{123}(d_1, d_2, d_3; \kappa_4) \sqrt[3]{d_1 d_2 d_3}. \quad (29)$$

Here, the functions g_{12} , g_{13} , and g_{23} can be specified in a manner similar to that of the interaction function g proposed in the current article, and the function g_{123} can be constructed as a polynomial function of the three combination doses. The last four terms in equation (29) are used to assess different patterns of three drug interactions. Indeed, when one of the drugs has a dose of zero, the index becomes a function of the other two drug

doses and the model falls into the specification of the model proposed in this article. When two of the drug doses become zero, the single index becomes the dose-response curve for a single drug, where monotonicity is preserved using the proposed monotonic single-index model. The proposed method uses penalized I-spline functions. Some other approach, such as using kernel splines and the pool adjacent violation algorithm, should be investigated to examine whether they perform better in estimating the index parameters and assessing drug interactions. The R code for the proposed monotonic single-index model is available in the Supplementary Material at the publisher's web-site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix: Specification of matrix D

The link function f in the proposed single-index model is estimated by the penalized residual sum of squares. The penalty $\int_L^U f''(u)^2 du$ can be written as the form of $\beta^T D \beta$, where D is a matrix with the first row and first column as zeroes and the rest as a $(K-3) \times (K-3)$ pentadiagonal matrix with the (j, j') entry as $\int_L^U [M_j^3(u)]' [M_{j'}^3(u)]' du$, $(1 \leq j, j' \leq K-3)$. We provide a detailed calculation for each entry in the $(K-3) \times (K-3)$ pentadiagonal matrix, denoted as $\tilde{D}$. Let us assume the knot sequence as $\{L = \tau_1 < \tau_2 < \dots < \tau_K = U\}$. Note that the (j, j') entry for matrix $\tilde{D}$ can be written as $\tilde{D}_{jj'} = \int_L^U [M_j^3(u)]' [M_{j'}^3(u)]' du$, where

$$\begin{aligned}
 M_j^3(z) &= \frac{3}{2(\tau_{j+3} - \tau_j)} [(z - \tau_j)M_j^2(z) + (\tau_{j+3} - z)M_{j+1}^2(z)] \\
 &= \frac{3(z - \tau_j)^2}{(\tau_{j+3} - \tau_j)(\tau_{j+2} - \tau_j)} M_j^1(z) \\
 &\quad + \left[\frac{3(z - \tau_j)(\tau_{j+2} - z)}{(\tau_{j+3} - \tau_j)(\tau_{j+2} - \tau_j)} + \frac{3(\tau_{j+3} - z)(z - \tau_{j+1})}{(\tau_{j+3} - \tau_j)(\tau_{j+3} - \tau_{j+1})} \right] M_{j+1}^1(z) \\
 &\quad + \frac{3(\tau_{j+3} - z)^2}{(\tau_{j+3} - \tau_j)(\tau_{j+3} - \tau_{j+1})} M_{j+2}^1(z).
 \end{aligned} \tag{30}$$

$$\begin{aligned}
M_j^{\prime 3}(z) &= \frac{6(z - \tau_j)}{(\tau_{j+3} - \tau_j)(\tau_{j+2} - \tau_j)} M_j^1(z) \\
&+ \left[\frac{3(\tau_j + \tau_{j+2} - 2z)}{(\tau_{j+3} - \tau_j)(\tau_{j+2} - \tau_j)} + \frac{3(\tau_{j+1} + \tau_{j+3} - 2z)}{(\tau_{j+3} - \tau_j)(\tau_{j+3} - \tau_{j+1})} \right] M_{j+1}^1(z) \\
&+ \frac{6(z - \tau_{j+3})}{(\tau_{j+3} - \tau_j)(\tau_{j+3} - \tau_{j+1})} M_{j+2}^1(z) \\
&= a_{j,1}(z - \tau_j) M_j^1(z) + (a_{j,2}z + a_{j,3}) M_{j+1}^1(z) + a_{j,4}(z - \tau_{j+3}) M_{j+2}^1(z), \quad (31)
\end{aligned}$$

with

$$\begin{aligned}
a_{j,1} &= \frac{6}{(\tau_{j+3} - \tau_j)(\tau_{j+2} - \tau_j)}, \\
a_{j,2} &= \frac{-6}{\tau_{j+3} - \tau_j} \left(\frac{1}{\tau_{j+2} - \tau_j} + \frac{1}{\tau_{j+3} - \tau_{j+1}} \right), \\
a_{j,3} &= \frac{3}{\tau_{j+3} - \tau_j} \left(\frac{\tau_{j+2} + \tau_j}{\tau_{j+2} - \tau_j} + \frac{\tau_{j+3} + \tau_{j+1}}{\tau_{j+3} - \tau_{j+1}} \right), \\
a_{j,4} &= \frac{6}{(\tau_{j+3} - \tau_j)(\tau_{j+3} - \tau_{j+1})}. \quad (32)
\end{aligned}$$

Therefore, for $1 \leq j \leq K-3$, we have

$$\begin{aligned}
\tilde{D}_{jj} &= \int_L^U [M_j^{\prime 3}(z)]^2 dz = \int_{\tau_j}^{\tau_{j+3}} [M_j^{\prime 3}(z)]^2 dz \\
&= \int_{\tau_j}^{\tau_{j+1}} [a_{j,1}(z - \tau_j) M_j^1(z)]^2 dz + \int_{\tau_{j+1}}^{\tau_{j+2}} [(a_{j,2}z + a_{j,3}) M_{j+1}^1(z)]^2 dz \\
&+ \int_{\tau_{j+2}}^{\tau_{j+3}} [a_{j,4}(z - \tau_{j+3}) M_{j+2}^1(z)]^2 dz \\
&= \frac{a_{j,1}^2(\tau_{j+1} - \tau_j)}{3} + \frac{a_{j,2}^2(\tau_{j+2}^2 + \tau_{j+1}^2 + \tau_{j+2}\tau_{j+1}) + 3a_{j,2}a_{j,3}(\tau_{j+2} + \tau_{j+1}) + 3a_{j,3}^2}{3(\tau_{j+2} - \tau_{j+1})} \\
&+ \frac{a_{j,4}^2(\tau_{j+3} - \tau_{j+2})}{3}. \quad (33)
\end{aligned}$$

For $1 \leq j \leq K-4$, we have

$$\begin{aligned}
\tilde{D}_{j,j+1} &= \tilde{D}_{j+1,j} \int_L^U M_j^3(z) M_{j+1}^3(z) dz = \int_{\tau_{j+1}}^{\tau_{j+3}} M_j^3(z) M_{j+1}^3(z) dz \\
&= \int_{\tau_{j+1}}^{\tau_{j+2}} [(a_{j,2}z + a_{j,3}) M_{j+1}^1(z)] [a_{j+1,1}(z - \tau_{j+1}) M_{j+1}^1(z)] dz \\
&\quad + \int_{\tau_{j+2}}^{\tau_{j+3}} [a_{j,4}(z - \tau_{j+3}) M_{j+2}^1(z)] [(a_{j+1,2}z + a_{j+1,3}) M_{j+2}^1(z)] dz \\
&= \frac{a_{j+1,1}[a_{j,2}(2\tau_{j+2} + \tau_{j+1}) + 3a_{j,3}]}{6} + \frac{-a_{j,4}[a_{j+1,2}(2\tau_{j+2} + \tau_{j+3}) + 3a_{j+1,3}]}{6}. \quad (34)
\end{aligned}$$

For $1 \leq j \leq K-5$, we have

$$\begin{aligned}
\tilde{D}_{j,j+2} &= \tilde{D}_{j+2,j} = \int_L^U M_j^3(z) M_{j+2}^3(z) dz = \int_{\tau_{j+2}}^{\tau_{j+3}} M_j^3(z) M_{j+2}^3(z) dz \\
&= \int_{\tau_{j+2}}^{\tau_{j+3}} [a_{j,4}(z - \tau_{j+3}) M_{j+2}^1(z)] [a_{j+2,1}(z - \tau_{j+2}) M_{j+2}^1(z)] dz \\
&= \frac{-a_{j,4}a_{j+2,1}(\tau_{j+3} - \tau_{j+2})}{6}. \quad (35)
\end{aligned}$$

Note that the support interval for $M_j^3(z)$ is $[\tau_j, \tau_{j+3}]$, and $M_{j'}^3(z)$ is zero outside the support interval. $M_j^3(z)$ and $M_{j'}^3(z)$ do not have a common support interval if $|j - j'| \geq 3$, which implies that $\tilde{D}_{j,j'} = \tilde{D}_{j',j} = 0$ if $|j - j'| \geq 3$. Therefore, $\tilde{D}$ is a $(K-3) \times (K-3)$ pentadiagonal matrix.

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Figure 1.

Results from the simulation study: A1) contour plot of the true underlying dose-response surface $f(d_1, d_2)$; A2) contour plot of the fitted dose-response surface based on the first simulated data with $\sigma = 1$; A3–A6) underlying dose-response profile curve (solid lines), the fitted dose-response profile curve along with its 95% confidence bounds based on the first simulated data (thick dashed lines), and the 10 fitted dose-response profile curves based on the first 10 simulated data (dotted lines) for each drug 2 dose level fixed at 0, 0.5, 1, and 2, respectively. B1) contour plot of the underlying interaction function $g(d_1, d_2)$; B2) contour

plot of the fitted interaction function $g(d_1, d_2)$ along with the contour plot of level zero for the upper 95% confidence surface and lower 95% confidence surface based on the first simulated data, which forms the shaded area; B3–B6) underlying dose-response profile function g (solid lines), fitted profile function g along with its 95% confidence bounds based on the first simulated data (thick dashed lines), and the 10 fitted function g based on the first 10 simulated data (dotted lines) for each drug 2 dose level fixed at 0, 0.5, 1, and 2, respectively.

Figure 2.

A1) contour plot of the observed growth inhibition on the lower 6×6 factorial design points; A2) contour plot of the fitted response surface at the same combination doses; B1) observed response (% inhibition) versus the indices (circles) and fitted responses versus indices (solid line); B2 and B3) observed values versus drug A doses (circles) and fitted $\hat{f}$ values versus drug A doses (lines) when drug A was applied alone (B2), and when equal amounts of drug A and drug B were combined (B3); C) contour plot of the polynomial

function $g(d_1, d_2; \kappa)$ along with the zero contour line of the 95% point-wise confidence surfaces for the function $g(d_1, d_2; \kappa)$.

Table 1

Simulation results based on 1000 simulated data, which were generated from the underlying response surface model (27) and fitted using the proposed single-index model, the underlying true model (27), and the linear model (i.e., misspecified model), respectively. “Truth” indicates the underlying parameter; “Est” indicates the mean of the 1000 estimates; “SE.emp” indicates the standard deviation of the 1000 estimates; “SE” indicates the mean of the 1000 standard errors based on each model.

	Single-index model				True model				Misspecified model			
	Truth	Est	SE.emp	SE	Est	SE.emp	SE	Est	SE.emp	SE		
$\alpha = 1$												
α_1	1.0	1.002	0.020	0.015	1.000	0.010	0.010	1	0.006	0.082		
α_2	0.9	0.944	0.207	0.145	0.902	0.077	0.072	7.456	0.062	0.910		
α_3	0.0	-0.046	0.245	0.176	0.000	0.106	0.099	-4.142	0.045	0.673		
α_4	0.0	-0.062	0.259	0.177	0.000	0.104	0.096	-4.141	0.054	0.787		
α_5	-0.3	-0.287	0.097	0.075	-0.301	0.047	0.043	0.088	0.014	0.208		
α_6	-0.3	-0.282	0.100	0.075	-0.301	0.043	0.041	0.087	0.014	0.210		
α_7	0.0	0.022	0.143	0.110	0.002	0.046	0.042	1.793	0.013	0.187		
$\sigma = 0.1$												
α_1	1.0	1.000	0.002	0.002	1.000	0.001	0.001	1.000	0.001	0.082		
α_2	0.9	0.890	0.018	0.016	0.900	0.010	0.010	7.453	0.008	0.908		
α_3	0.0	0.007	0.020	0.018	0.000	0.015	0.013	-4.140	0.006	0.671		
α_4	0.0	0.006	0.021	0.018	0.000	0.014	0.013	-4.141	0.007	0.786		
α_5	-0.3	-0.303	0.009	0.008	-0.300	0.006	0.006	0.087	0.002	0.207		
α_6	-0.3	-0.302	0.009	0.008	-0.300	0.006	0.005	0.087	0.002	0.209		
α_7	0.0	0.001	0.018	0.016	0.001	0.006	0.006	1.793	0.002	0.187		

Table 2

Simulation results for the estimated response surface function f and interaction function g based on the 1000 simulated data, which were generated from the underlying response surface model (27) and fitted using the proposed single-index model. “Truth” indicates the values based on the underlying data generating model (27); “Est” indicates the mean of the 1000 estimates; “SE.emp” indicates the standard deviation of the 1000 estimates; “SE” indicates the mean of the 1000 estimated standard errors at each combination dose.

Dose	Response surface function f						Interaction function g					
	$\alpha = 1$			$\sigma = 0.1$			$\alpha = 1$			$\sigma = 0.1$		
	Truth	Est	SE	SE.emp	Est	SE	Truth	Est	SE	SE.emp	Est	SE
(0.0, 0.0)	0.0	0.2	0.57	0.38	0.0	0.06	0.04	0.90	0.17	0.14	0.89	0.02
(0.1, 0.0)	9.1	9.0	0.36	0.29	9.1	0.04	0.03	0.87	0.13	0.11	0.86	0.01
(0.5, 0.0)	33.3	33.3	0.41	0.36	33.3	0.04	0.04	0.75	0.11	0.09	0.74	0.01
(1.0, 0.0)	50.0	49.9	0.41	0.35	50.0	0.04	0.04	0.60	0.12	0.09	0.60	0.01
(2.0, 0.0)	66.7	66.6	0.39	0.34	66.7	0.04	0.04	0.30	0.19	0.12	0.30	0.02
(4.0, 0.0)	80.0	79.9	0.52	0.38	80.0	0.04	0.04	-0.30	0.34	0.19	-0.30	0.03
(0.0, 0.1)	9.1	9.1	0.36	0.29	9.1	0.04	0.03	0.87	0.13	0.10	0.86	0.01
(0.1, 0.1)	22.1	22.1	0.49	0.39	22.1	0.05	0.04	0.84	0.09	0.07	0.83	0.01
(0.5, 0.1)	43.2	43.2	0.38	0.32	43.2	0.04	0.03	0.72	0.06	0.05	0.72	0.01
(1.0, 0.1)	56.1	56.2	0.38	0.32	56.2	0.04	0.03	0.57	0.07	0.06	0.57	0.01
(2.0, 0.1)	69.0	69.0	0.35	0.32	68.9	0.03	0.03	0.27	0.12	0.07	0.27	0.01
(4.0, 0.1)	79.6	79.5	0.32	0.30	79.5	0.03	0.03	-0.33	0.24	0.13	-0.33	0.02
(0.0, 0.5)	33.3	33.4	0.43	0.36	33.4	0.04	0.04	0.75	0.10	0.08	0.74	0.01
(0.1, 0.5)	43.2	43.2	0.38	0.32	43.2	0.04	0.03	0.72	0.06	0.05	0.72	0.01
(0.5, 0.5)	56.5	56.5	0.32	0.29	56.5	0.03	0.03	0.60	0.04	0.04	0.60	0.01
(1.0, 0.5)	64.5	64.5	0.34	0.32	64.5	0.03	0.03	0.45	0.05	0.04	0.45	0.00
(2.0, 0.5)	72.6	72.6	0.33	0.31	72.6	0.03	0.03	0.15	0.07	0.05	0.15	0.01
(4.0, 0.5)	79.4	79.5	0.38	0.32	79.4	0.03	0.03	-0.45	0.15	0.08	-0.45	0.01
(0.0, 1.0)	50.0	50.0	0.41	0.35	50.0	0.04	0.04	0.60	0.11	0.09	0.60	0.01
(0.1, 1.0)	56.1	56.2	0.35	0.32	56.2	0.04	0.03	0.57	0.06	0.05	0.57	0.01
(0.5, 1.0)	64.5	64.5	0.34	0.32	64.5	0.03	0.03	0.45	0.05	0.04	0.45	0.01
(1.0, 1.0)	69.7	69.7	0.42	0.35	69.7	0.04	0.04	0.30	0.05	0.04	0.30	0.01

Dose	Response surface function f						Interaction function g					
	$\alpha = 1$			$\sigma = 0.1$			$\alpha = 1$			$\sigma = 0.1$		
	Truth	Est	SE.emp	SE	Est	SE	Truth	Est	SE.emp	SE	Est	SE
(2.0, 1.0)	75.0	75.0	0.33	0.30	75.0	0.03	0.00	0.01	0.06	0.05	0.00	0.01
(4.0, 1.0)	79.2	79.1	0.39	0.34	79.2	0.04	-0.60	-0.58	0.10	0.06	-0.60	0.01
(0.0, 2.0)	66.7	66.7	0.40	0.34	66.7	0.04	0.30	0.33	0.18	0.12	0.30	0.02
(0.1, 2.0)	69.0	69.0	0.36	0.31	68.9	0.04	0.27	0.29	0.12	0.07	0.27	0.01
(0.5, 2.0)	72.6	72.6	0.33	0.31	72.6	0.03	0.15	0.16	0.08	0.05	0.15	0.01
(1.0, 2.0)	75.0	75.0	0.34	0.30	75.0	0.03	0.00	0.01	0.06	0.05	0.00	0.01
(2.0, 2.0)	77.3	77.3	0.37	0.33	77.3	0.04	-0.30	-0.30	0.06	0.05	-0.30	0.01
(4.0, 2.0)	77.6	77.5	0.46	0.38	77.5	0.04	-0.90	-0.89	0.06	0.04	-0.90	0.01
(0.0, 4.0)	80.0	80.0	0.52	0.40	80.0	0.04	-0.30	-0.23	0.34	0.19	-0.30	0.03
(0.1, 4.0)	79.6	79.5	0.34	0.30	79.5	0.03	-0.33	-0.28	0.26	0.13	-0.33	0.03
(0.5, 4.0)	79.4	79.5	0.38	0.32	79.4	0.03	-0.45	-0.42	0.17	0.08	-0.45	0.02
(1.0, 4.0)	79.2	79.1	0.39	0.34	79.2	0.04	-0.60	-0.58	0.12	0.06	-0.60	0.01
(2.0, 4.0)	77.6	77.6	0.48	0.38	77.5	0.04	-0.90	-0.89	0.07	0.04	-0.90	0.01
(4.0, 4.0)	66.7	66.7	0.59	0.43	66.7	0.06	-1.50	-1.50	0.02	0.02	-1.50	0.01