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RESEARCH PAPER

Application of Nonlinear Fitting and Selection of the Most Fitted Equation by AIC in Stability Test of Pharmaceutical Ingredients

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ABSTRACT

We proposed a method for objectively selecting the most fitted rate equation among candidate rate equations based on chemical kinetics by both nonlinear regression analysis and Akaike's information criterion (AIC), to express the decrease of pharmaceutical ingredient as an appropriate equation. Pseudo-zero, first, and second-order rate equations were prepared as candidates beforehand, and AIC was introduced for selecting the most fitted rate equation among the candidates. We compared the proposed method to the Weibull method that expressed any decrease patterns as a single equation.

We quantified the contents of thiamine nitrate (VB1) and taurine after storage for one, three, and six months under 40°C–75% relative humidity by high-pressure liquid chromatography. Decrease patterns of each sample were most fitted to one of the candidate rate equations, that is, pseudo-zero, first, and second-order rate equations, respectively, and the degree of fit in the most fitted equation was superior to that of the Weibull method, except for the pseudo-first-order rate equation.

Moreover, we confirmed that the proposed method was considerably precise for estimating the stability of pharmaceuticals by comparing estimated residual rates and confidence intervals to experimental data.

Key Words: *Nonlinear fitting; AIC; Stability test; Shelf-life; Prediction; Pharmaceuticals*

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INTRODUCTION

It is important to presume shelf-life of pharmaceutical ingredients with statistical accuracy on the basis of results of a stability test (long-term or accelerated). In this case, the apparent decrease pattern of the object ingredient is generally expressed as a pseudo rate equation, because it is very difficult to express the complex reaction mechanisms (combination of elementary reactions) in multi-compound formulations.[1]

When the shelf-life is estimated from results of experimental stability tests, linear regression analysis such as the method of Woolfe and Worthington[2] has long been used. However, not only a linear decrease in ingredient content but also a nonlinear decrease is often seen in stability tests. When linear regression analysis is applied to the nonlinear decrease, the degree of fit is poor and the confidence interval is non-symmetrical and broadened excessively.[3–5]

When nonlinear regression analysis is applied to the nonlinear decrease, the degree of fit is improved and the confidence interval becomes narrow.[3,4] With such a viewpoint, the application of nonlinear regression analysis has been examined to estimate the stability of pharmaceutical ingredients,[1,4,6] and whether a single model equation can express any decrease pattern has been investigated.[1,6]

In particular, the Weibull method[7] is widely used as a statistical method useful for appropriately showing any decrease patterns, both linear and nonlinear. However, the degree of fit in the Weibull method was inferior to that in a rate equation such as pseudo-zero and second-order rate equations, excepting the pseudo-first-order rate equation.[6]

We therefore proposed a method to select the most fitted rate equation objectively among candidate model equations with the use of the Akaike's information criterion (AIC) method,[8–10] instead of using a single model equation such as in the Weibull method. In this proposed method, the candidate model equations were prepared beforehand, based on chemical kinetics (e.g., pseudo-zero, first, and second-order reaction), and AIC was applied for selecting the most fitted rate equation among candidate rate equations.

Eventually, the degree of fit was compared between this proposed method and the Weibull method by using experimental stability data to verify the validity of this proposed method.

THEORETICAL

Maximum Likelihood Estimation in the Pseudo Rate Equation

In general, decrease patterns can be expressed by the following equations of pseudo-zero-order type (Eq. 1), pseudo-first-order type (Eq. 2), and pseudo-second-order type (Eqs. 3 and 4), when the contents of the object ingredients are plotted against time. In this article, the four rate equations (Eqs. 1–4) are enumerated. Though Okusa[6] used Eq. (4) as a pseudo-second-order rate equation indicating the reaction between two identical molecules in a numerical study, we added Eq. (3) indicating the reaction between two different molecules.

Equations (1)–(4) are integrated into Eqs. (5)–(8), respectively:

$$-\frac{dA}{dt}=k \quad (1) \quad \xrightarrow[integrated]{} \quad A=A_0-kt \tag{5}$$

$$-\frac{dA}{dt}=kA \quad (2) \quad \xrightarrow[integrated]{} \quad A=A_0e^{-kt} \tag{6}$$

$$\frac{dP}{dt}=kAB \quad (3) \quad \xrightarrow[integrated]{} \quad \frac{1}{A_0-B_0}\ln\frac{AB_0}{A_0B}=kt \tag{7}$$

$$-\frac{1}{2}\frac{dA}{dt}=kA^2 \quad (4) \quad \xrightarrow[integrated]{} \quad A=\frac{A_0}{2ktA_0+1} \tag{8}$$

where k is the kinetic rate constant and t is time. A_0 and B_0 are contents of the ingredients at time 0, and A and B are those at time t, respectively. P is the content of the product of the reaction at time t.

Moreover, Eq. (7) can be transformed into Eq. (9):

$$A=\frac{(A_0-B_0)\times(A_0/B_0)\times e^{(A_0-B_0)\times kt}}{(A_0/B_0)\times e^{(A_0-B_0)\times kt}-1} \tag{9}$$

Substituting the data from the stability test into Eqs. (5), (6), (9), and (8), values of each parameter and the residual variance in each rate equation were calculated by nonlinear regression analysis according to the simplex method and Newton–Raphson method. All of the calculations described above were carried out with SAS programs.

Comparison of the Degree of Fit Among the Rate Equations by AIC[8–10]

The maximum logarithm likelihood (MLL) can be obtained to calculate a maximum likelihood

estimate of parameters and residual mean square in a model equation. The AIC is defined by:

$$\mathrm{AIC} = -2\mathrm{MLL} + 2k \tag{10}$$

where k is the number of parameters in the equation.

Thus, the AIC contains intrinsically the residual mean square derived from the Gauss criterion. Moreover, the AIC method can compare the degree of fit among rate equations having different order by correcting the number of parameters.

The number k is limited to be less than $2\sqrt{n}$ (where n is the number of data), because the AIC can't be set up on statistics when the number k is excessively large.

On the other hand, the MLL of the polynomial can be expressed by the following equation:

$$\mathrm{MLL} = -\frac{n}{2} \times \log 2\pi - \frac{n}{2} \times \log(V_r) - \frac{n}{2} \tag{11}$$

where V_r is residual variance.

The AIC of the polynomial can be summarized from Eqs. (10) and (11) as follows:

$$\mathrm{AIC} = n \log 2\pi + n \log(V_r) + n + 2k \tag{12}$$

The equation indicating the least AIC value among candidate equations is selected as the most fitted equation. Moreover, a lower order equation should be selected when the AIC values are equal among equations.

EXPERIMENTAL

The samples, six model formulations of liquid pharmaceuticals (multi-compound formulations which contained several vitamins, taurine, herbal medicines, and other agents) in glass bottles, were prepared as homogeneous systems.

Model Formulations (Prescriptions)

Model formulations used in the stability test are shown in Table 1. The contents of thiamine nitrate (VB1) or derivatives of VB1 are quantified in samples A, B, C, and D, and the contents of taurine are quantified in samples E and F.

Storage Conditions

These samples were stored for various periods (one, three, six, and eight months) under accelerated stability test conditions (40°C–75% relative humidity).

Measurements

After diluting the sample to a suitable concentration, the content of VB1 (or derivative of VB1) was quantified by the high-pressure liquid chromatography (HPLC) system with a C18 column. The eluent was a mixture of water containing SDS (sodium dodecyl sulfate) (0.5% v/v), acetonitrile, and phosphoric acid (mixture ratio of the eluent is 530:470:1). The chromatogram was monitored at a wavelength of 260 nm.

Table 1

Model Formulations in Experimental Samples

Formulation	VB1[a]	Derivative of VB1	Taurine	VB2[b], VB6[c]	Caffeine	Others (Amino Acid, Metal, etc.)	Herbal Medicine
A	●	—	○	○	○	○	—
B	—	●	○	○	○	○	—
C	●	—	○	○	○	○	○
D	●	—	○	○	○	○	○
E	—	—	●	○	○	—	○
F	—	—	●	○	—	○	○

●, Measured ingredient. ○, Other compounding ingredient.
[a]VB1, thiamine nitrate.
[b]VB2, riboflavin sodium phosphate.
[c]VB6, pyridoxine hydrochloride.

After diluting the sample to a suitable concentration, taurine was quantified by the HPLC system with an ion exchange column. The eluent was diluted perchloric acid (0.0167 mol/L). The chromatogram was monitored with a refractive index detector.

The contents are shown as the residual rate (%) vs. initial content.

RESULTS

Statistical analysis was performed as described in the theoretical section to estimate the most fitted equation among candidate equations to the decrease pattern.

In Table 2, various decrease patterns of VB1 were obtained from experimental data because of the difference in the formulations (i.e., coexistent ingredients, pH, etc.). On statistical analysis, the decrease patterns of VB1 in samples A, B, C, and D were the most fitted to pseudo-zero-order (A), pseudo-first-order (B), and pseudo-second-order [different bimolecular type (C) and identical bimolecular type (D)] rate equations among candidate rate equations, respectively.

On the other hand, the linear and nonlinear decrease patterns for taurine were obtained from experimental data because of the difference in the formulations as well as VB1. The decrease patterns of taurine were the most fitted to pseudo-zero-order (E) and pseudo-second-order [different bimolecular type (F)] rate equations among candidate rate equations, respectively.

Though the Weibull equation fitted all decrease patterns relatively well, we demonstrated experimentally that the degree of fit in one of the rate equations (pseudo-zero and second-order rate equations, excepting pseudo-first-order rate equation) was superior to that in the Weibull equation, as reported in a numerical study by Okusa.[6] In particular, the pseudo-second-order (different molecular type) equations selected as the most fitted equation were fitted much better than the Weibull equation, based on AIC values in cases C and F.

Then, the validity of selected equations by the proposed method were verified with experimental data for C and F.

Estimated residual rates and confidence intervals at eight months were calculated from zero to six-month data in each rate equation (pseudo-zero, second-order, and Weibull equations), and the results are shown in Table 3. The curves of these rate equations are shown in Figs. 1 and 2. Pseudo-zero-order rate equations are shown in Figs. 1a and 2a, in which the confidence intervals of these rate equations were broadened excessively more than that of the most fitted rate equation (pseudo-second-order rate equation) among candidate rate equations. Moreover, several data points at eight months were beyond the confidence intervals in Figs. 1a and 2a. The confidence intervals were narrower in descending order of AIC value [(b) < (c) < (a)]. Because the confidence intervals (at eight months) for the pseudo-second-order rate equation selected as the most fitted equation with the least AIC value were

Table 2

Values of AIC in Each Equation

Formulation	Ingredient	pH	Reduction (%)[a]	Value of AIC				
						Second-Order[b]		Weibull
				Zero-Order[b]	First-Order[b]	Different	Identical	
A	VB1	2.5	8.1	55.2*	55.2	58.3	55.5	55.3
B	Derivative of VB1	2.8	10.2	86.3	86.0*	89.0	86.0	86.0
C	VB1	2.5	8.0	116.5	114.7	64.9*	112.9	77.6
D	VB1	3.0	16.0	80.2	75.2	76.3	72.5*	73.8
E	Taurin	4.6	8.3	70.8*	71.1	75.8	71.5	71.2
F	Taurin	3.0	4.1	126.0	125.6	86.6*	125.2	95.8

[a]The amount degraded after six months.
[b]Pseudo rate equation based on chemical reaction.
*Equation selected by proposed method.

Table 3

Estimated Value and Confidence Interval (from Data for Zero to Six Months) and Experimental Values at Eight Months

Formulation	Zero-order[a]		First-order[a]		Second-order[a]						Experimental Value[b]
					Different		Identical		Weibull		
	Estimated Value	Confidence Interval	Estimated Value	Confidence Interval	Estimated Value	Confidence Interval	Estimated Value	Confidence Interval	Estimated Value	Confidence Interval	
C	89.2	±2.5	89.3	±2.4	91.9	±1.2	89.4	±2.3	90.9	±1.5	91.6 (0.3)
F	94.0	±2.8	94.0	±2.8	95.7	±1.5	94.0	±2.8	95.2	±1.8	95.6 (0.6)

[a]Pseudo rate equation based on chemical reaction.
[b]Values in parentheses indicate relative standard deviation (%).

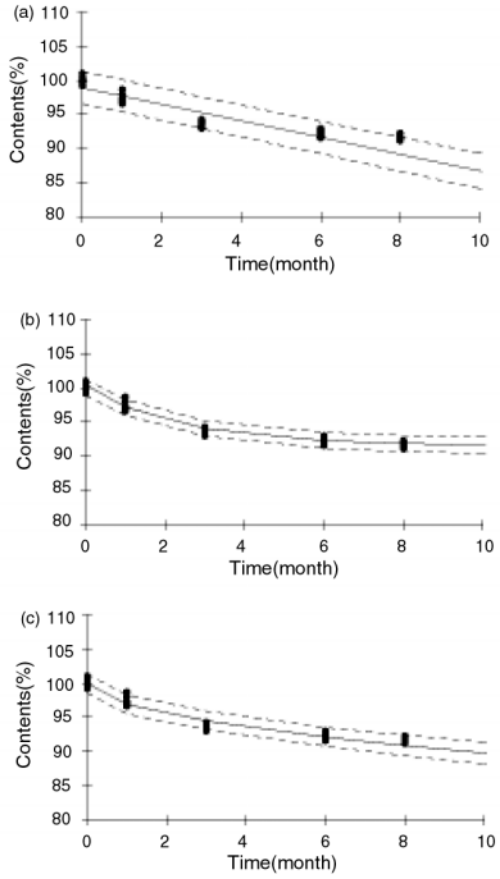

Figure 1. Curve of each equation and confidence interval to decrease pattern (C): (a) zero-order rate equation; (b) second-order rate equation; (c) Weibull equation.

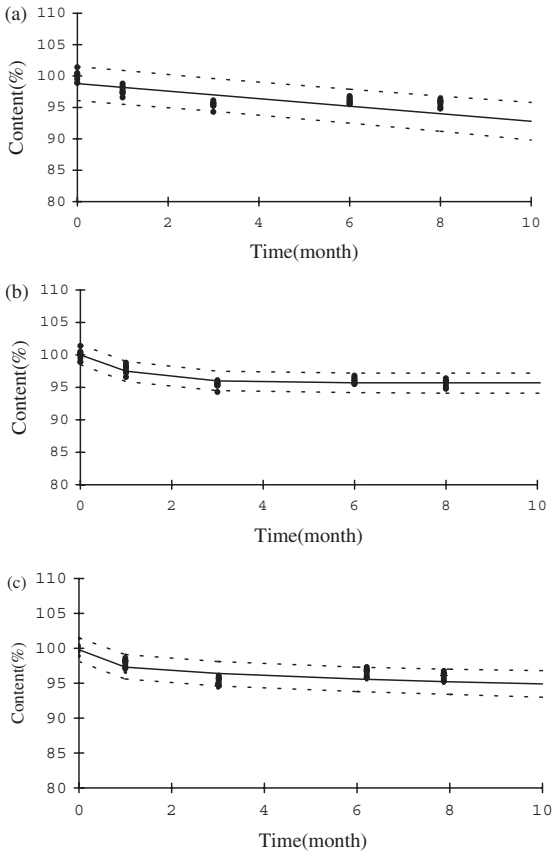

Figure 2. Curve of each equation and confidence interval to decrease pattern (F): (a) zero-order rate equation; (b) second-order rate equation; (c) Weibull equation.

the narrowest among these rate equations containing the Weibull equation, this proposed method was superior to the Weibull method in estimating the stability of pharmaceuticals.

For these results, since the most fitted rate equation among candidate rate equations could estimate the residual rate at eight months more precisely, this method was verified to be excellent for estimating the stability of pharmaceutical ingredients.

DISCUSSION

When the experimental stability data were expressed as rate equations, linear regression analysis such as the Woolfe method is often used because of its simplicity. For the nonlinear decrease, however, the degree of fit is poor and the confidence interval is broadened excessively. In this article, similar samples are shown in Figs. 1a and 2a.

Okusa[6] showed that in a numerical study pseudo-zero or second-order rate equations were more fitted than the Weibull equation. However, (i) it is very complicated to apply experimental data to a lot of rate equations, and (ii) it is difficult to compare the degree of fit among rate equations with different parameters. Therefore, Okusa reported the utility of the Weibull method that expressed any decrease pattern as a single equation, although its degree of fit was inferior to that in each rate equation, such as pseudo-zero or second-order rate equations.

We therefore proposed a method of evaluation of pharmaceutical stability that could solve the above problems, considering it appropriate to select the most fitted equation among candidate rate equations based on chemical kinetics for both linear and

nonlinear decrease patterns. A computer with improved hardware and software technology solved the complication of calculating, and the AIC method solved the difficulties of selecting the most fitted equation among candidate rate equations. By these means, we developed a method to calculate the residual variances and compared rate equations having different order by correcting parameters with the AIC method.

The proposed method was superior to the Weibull method in the degree of fit (Tables 2 and 3, Figs. 1 and 2). In particular, when the pseudo-second-order rate equation (different bimolecular type) was selected as the most fitted equation among candidate rate equations, the degree of fit in the equation was much better than that in the Weibull method. Therefore, four rate equations including Eq. (3) (different bimolecular reaction) were considered appropriate as candidates.

Though the linear fitting has been conventionally used in case of less than 10% degradation, we could confirm that the formulations showing less than 10% degradation, for example, C (8.0%) and F (4.1%), fitted mostly to the nonlinear rate equation in Figs. 1 and 2. These results indicate that the proposed method has the ability to distinguish the linear or nonlinear decrease pattern, even with less than 10% degradation. Moreover, because the most fitted rate equation among candidate rate equations could predict the subsequent stability with narrower confidence intervals, this proposed method is considerably precise for estimating shelf-life.

Consequently, we developed an improved statistical evaluation method to estimate pharmaceutical stability and indicated that the proposed method was more accurate than conventional methods such as the Weibull method.

Though we prepared four fundamental rate equations as the candidates in this article, it was possible to add more complicated rate equations for applying solid pharmaceuticals as a heterogeneous system.

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