distribution was assumed to be Gaussian, a small fraction of the population would be represented by the values of elimination rate constants at the extreme of the range. Very few individuals would be subjected to the maximum error of the method. Figs. 4 and 5 represent error patterns of specific cases for certain values of K and k_m; they have not been constructed to represent general cases. For any given case, similar plots should be constructed to evaluate error.

DISCUSSION

The optimum sampling time for determining the maintenance dose of parent drug required to give a desired steady-state concentration of metabolite for the drug–metabolite pair imipramine–desipramine indicated by this analysis is 48 hr. A linear relationship between the log of the 24-hr concentration of desipramine following the first dose of imipramine and the log of the eventual steady-state concentration of the drug, if the same dose is kept constant and administered daily, has been found clinically (6). The mathematical basis for relating these two concentrations arises from a rearrangement of Eq. 6 that gives rise to a proportionality factor with the general behavior of Ψ_m (10). The results of the present analysis suggest that the relationship between concentration at 24 hr and eventual steady-state concentration would be curvilinear. A log–log transformation might linearize such a plot. It is expected that a sample collected 48 hr after the first dose would appear to be linearly related to the eventual steady-state concentration.

There are two critical considerations in applying this method to any drug–metabolite pair: (*a*) the error which will be encountered as a function of the elimination kinetics of the pair and (*b*) the possibility that the elimination kinetics may dictate a value of t^* that is not clinically feasible. The variability of Ψ_m is a function of the elimination kinetics of the pair in the population and cannot be overcome when single-point prediction schemes are used. Thus, a poor estimate of maintenance dose will always be obtained for some fraction of the population. When the optimum value of t^* is not used because it is too short to be clinically convenient and a longer time is adopted, the error of the method increases but in a somewhat conservative manner. Patients who eliminate the drug and metabolite slowly will tend to be underdosed and those who eliminate the drug quickly (requiring a relatively larger maintenance dose) tend to be overdosed. This situation is perhaps more tolerable than the converse: when a t^* shorter than the optimum is adopted, patients who eliminate the drug most slowly will tend to be overdosed and those who eliminate it more quickly will tend to be underdosed. Another observation may be more to the point: when the optimum value of t^* is not used, the relationship between $1/D_m$ and C^*_m will become less well-characterized by a straight line. If the curvilinear nature of the relationship can be taken into account, reasonably accurate dose prediction may still be possible.

Single-point dose prediction methods appear to be applicable to most drugs and their metabolites. However, the optimum sampling time for the dose required to give a desired steady-state concentration of the parent drug may be quite different than that required to give a target metabolite concentration. If dosage prediction is warranted for a particular drug (10) and the kinetics of the drug are linear, it appears likely that a single-point method could be developed to suit using the techniques described here. However, it must be remembered that the predicted dose is an estimate that must be confirmed by obtaining samples at steady state.

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Bayesian Approach to Bioequivalence Assessment: An Example

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Received February 22, 1982, from the **Scientific Computing Centre, CIBA-GEIGY AG, Basel, Switzerland;* ‡*Essex Chemie AG, Luzern, Switzerland;* and §*Rheinisch-Westfälische TH, Aachen, West Germany.* Accepted for publication August 26, 1982.

Abstract □ The statistical methods required for a Bayesian analysis of bioequivalence are outlined and numerically illustrated. The analysis consists of the calculation of the posterior probability, given the experimental results, that the ratio of true means of a new and a standard formulation of a drug with respect to some biological response lies in a given interval. Nomograms helpful for the calculation of these probabilities are provided.

Keyphrases □ Bioequivalence—assessment by Bayesian analysis, statistical methods, example and nomograms □ Bayesian analysis—bioequivalence assessment, statistical methods, example, and nomograms

Comparative bioavailability studies serve to investigate the pharmaceutical properties of two or more formulations of the same drug. Decisions on whether two formulations are bioequivalent are usually made by comparing biological responses such as area under the plasma concentration curve or the maximum peak concentration. Since in many instances the objective of a bioavailability study is not to show a difference between formulations, but rather to investigate whether any difference is of practical importance, Westlake (1) and Metzler (2) suggest that hypothesis tests of no difference are of little value.

In this paper the statistical methods needed to perform a Bayesian analysis of bioequivalence given by Mandallaz and Mau (3) are outlined. This method has been illustrated

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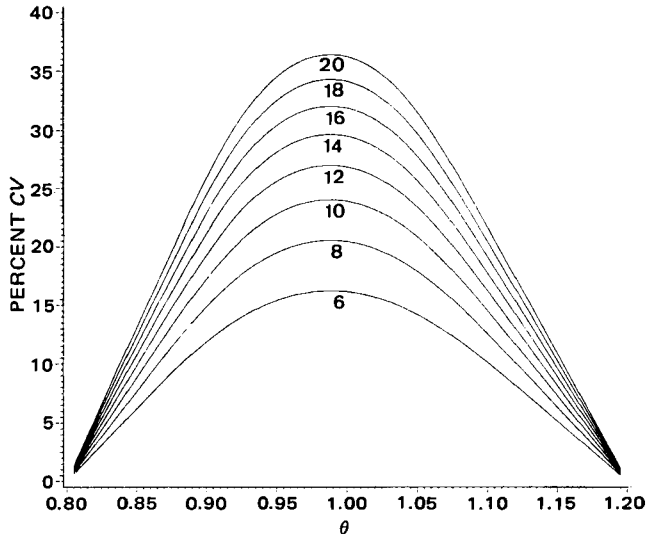

Figure 1—*Values of observed ratios and percent* CV *giving a posterior probability of 0.90 that the true ratio lies between 0.8 and 1.2 for various values of* n.

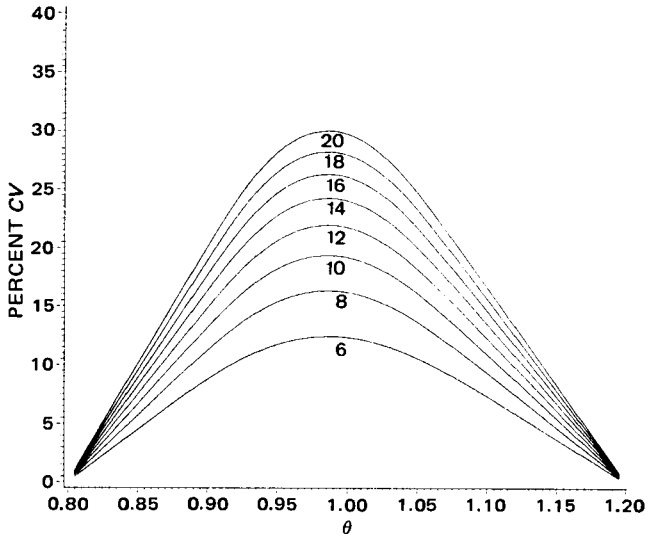

Figure 2—*Values of observed ratios and percent* CV *giving a posterior probability of 0.95 that the true ratio lies between 0.8 and 1.2 for various values of* n.

by Fluehler *et al.* (4) using historical data. The numerical calculations required are illustrated by an example. Readers interested in a general introduction to statistical considerations involved in bioequivalence assessment are referred to Metzler (2) or Westlake (1). An alternative Bayesian approach has been developed by Selwyn *et al.* (5).

THEORETICAL

It is assumed that a new formulation of a drug has been developed and that it is to be compared with the standard formulation of the drug. The comparison will focus on the area under the blood concentration curve (AUC) and the maximum concentration of that curve (C_{max}). If the ratio, θ, of the true means of the new to the standard formulation of the chosen measure (AUC or C_{max}) lies between given limits r_1 and r_2, that is:

$$r_1 \leq \theta \leq r_2 \qquad \text{(Eq. 1)}$$

then the formulations are said to be bioequivalent. The choice of r_1 and r_2 is made on medical and/or regulatory grounds. Thus, for AUC, one might set $r_1 = 0.8$ and $r_2 = 1.2$, which correspond to the AUC of the new formulation differing by at most ±20% from that of the standard formulation. It should be noted that r_1 and r_2 need not be the same for AUC and C_{max} and in general they need not be symmetric about 1.

To provide evidence for or against the bioequivalence statement given in Eq. 1, estimates of the true means of either AUC or C_{max} for both formulations and an estimate of their variance are needed. Because of the large intersubject variation with respect to the absorption, distribution, and elimination of a drug, the crossover design is usually considered the most appropriate for comparative bioavailability studies. In such a design each of n subjects receives each formulation with a sufficiently large time lag to ensure an adequate washout period. The statistical model for a two-way crossover design is:

$$x_{ijk} = \mu + \xi_i + \pi_j + \phi_k + \epsilon_{ijk} \qquad \text{(Eq. 2)}$$

where x_{ijk} are the observed values, μ is the overall mean, ξ_i is the ith subject effect ($i = 1, \ldots, n$), π_j is the jth period effect ($j = 1, 2$), ϕ_k is the kth formulation effect ($k = 1, 2$), and ϵ_{ijk} are experimental errors associated with the x_{ijk} values, which are assumed to be independently normally distributed with zero means and common variance σ^2.

The AUC values are assumed to be normally distributed according to the model in Eq. 2. The C_{max} values, however, are assumed to be log-normally distributed so that $\ln(C_{max})$, instead of C_{max}, obeys the aforementioned model. The analysis of variance (ANOVA) for the crossover design is outlined in basic statistical texts, *e.g.*, Cochran and Cox (6). The error mean square from the ANOVA gives an estimate of the experimental error variance.

A Bayesian approach (3, 4) is used to investigate bioavailability defined in Eq. 1. In this approach the posterior distribution of θ given the experimental results is obtained, from which the posterior probability that θ lies between r_1 and r_2 is calculated; if this probability is large enough, bioequivalence is accepted. The advantage of a Bayesian approach is that the whole distribution of θ may be examined either through the posterior distribution itself or by calculating the cumulative distribution function.

The posterior probability that θ lies between r_1 and r_2 is calculated by:

$$Pr(r_1 \leq \theta \leq r_2) = \int_B^A t_\nu(\tau)d\tau \qquad \text{(Eq. 3)}$$

where $t_\nu(\tau)$ is the density function of Student's t distribution with ν degrees of freedom (DF) and ν is the number of DF of the error mean square (MSQ) from the ANOVA. The integration limits for AUC, normally distributed data, are given by:

$$A = \frac{(\hat{\theta} - r_1)n^{1/2}}{CV(1 + r_1^2)^{1/2}} \qquad \text{(Eq. 4)}$$

$$B = \frac{(\hat{\theta} - r_2)n^{1/2}}{CV(1 + r_2^2)^{1/2}} \qquad \text{(Eq. 5)}$$

with

$$\hat{\theta} = \frac{\bar{X}_{NEW}}{\bar{X}_{STD}} \qquad \text{(Eq. 6)}$$

$$CV = \frac{S}{\bar{X}_{STD}} \qquad \text{(Eq. 7)}$$

$$S = \sqrt{\text{Error (MSQ) from ANOVA}} \qquad \text{(Eq. 8)}$$

$\bar{X}_{NEW}$ and $\bar{X}_{STD}$ are the observed arithmetic formulation means; n is the number of subjects. The integration limits for C_{max}, log-normally distributed data, are given by:

$$A = \frac{[\bar{X}_{NEW} - \bar{X}_{STD} - \ln(r_1)]n^{1/2}}{2^{1/2}S} \qquad \text{(Eq. 9)}$$

$$B = \frac{[\bar{X}_{NEW} - \bar{X}_{STD} - \ln(r_2)]n^{1/2}}{2^{1/2}S} \qquad \text{(Eq. 10)}$$

where now $\bar{X}_{NEW}$ and $\bar{X}_{STD}$ denote the observed arithmetic means of the log-transformed C_{max} values. The cumulative posterior distribution function $Pr(\theta < \theta_0)$, is calculated by setting $r_1 = 0$ and $r_2 = \theta_0$ in Eq. 3.

Alternative methods of displaying information from the posterior distribution are available. Fluehler *et al.* (4) show how Eq. 3 may be used to display posterior probabilities of given intervals in histogram form, while the posterior density function itself may be calculated from the results of Mandallaz and Mau (3).

Although the calculation of the posterior probabilities are straightforward, the nomograms shown in Figs. 1 and 2 are useful tools for the practitioner using the normal model (Eqs. 3–8). In these nomograms the bioequivalence range was chosen to be 0.8–1.2, although nomograms for

Table I—Comparative Bioavailability Study

Number of Subjects	Standard Formulation				New Formulation			
	Period	AUC	C_{max}	$\ln(C_{max})$	Period	AUC	C_{max}	$\ln(C_{max})$
1	2	144.57	296.11	5.6907	1	115.21	67.97	4.2190
2	1	98.17	146.69	4.9883	2	106.60	92.63	4.5286
3	1	121.87	259.37	5.5583	2	129.70	97.75	4.5824
4	2	30.20	197.36	5.2850	1	52.85	196.53	5.2808
5	2	131.51	281.37	5.6397	1	59.42	59.71	4.0895
6	1	104.17	179.14	5.1882	2	152.76	54.99	4.0072
7	1	71.54	251.37	5.5269	2	31.24	93.11	4.5337
8	2	71.98	233.29	5.4523	1	108.22	109.26	4.6938
9	2	78.83	173.61	5.1568	1	82.05	152.18	5.0251
10	1	140.48	227.56	5.4274	2	101.10	177.09	5.1767
11	2	75.27	211.85	5.3559	1	58.72	100.70	4.6121
12	1	111.56	225.71	5.4192	2	83.27	172.22	5.1488
Mean	—	98.35	223.62	5.3907	—	90.10	114.51	4.6581

Table II—ANOVA for the Observed AUC

Source	*DF*	SSQ	MSQ
Subject	11	19570.1	1779.1
Period	1	6.8	6.8
Formulation	1	408.4	408.4
Error	10	6904.2	690.4
Total	23	26889.5	—

Table III—ANOVA for the Observed $\ln(C_{max})$

Source	*DF*	SSQ	MSQ
Subject	11	1.0101	0.0918
Period	1	0.0117	0.0117
Formulation	1	3.2201	3.2201
Error	10	1.4661	0.1466
Total	23	5.7079	—

other ranges and for the log-normal model can be produced. The nomograms give the sample sizes required to achieve posterior probabilities of 0.90 and 0.95 as a function of the observed ratio and percentage coefficient of variation ($100 \times CV$). For instance, suppose an experiment with 12 subjects was carried out and an observed ratio of 1.1 with an observed coefficient of variation of 15% was obtained. From Fig. 2 it is seen that, with this data, a sample size of between 16 and 18 is necessary to achieve a posterior probability of 0.95. However Fig. 1 shows that 12 subjects yield, with the same data, a posterior probability >0.90.

RESULTS

The theoretical results presented are now illustrated by an example. Assume that a slow-release formulation of a drug (NEW) has been developed with the aim of producing markedly lower peak concentrations than the standard drug (STD), while at the same time delivering a similar amount of active ingredient to the circulation. Medical considerations, therefore, lead to the following conditions for bioequivalence:

$$\text{AUC: } 0.8 \le \theta \le 1.2 \qquad \text{(Eq. 11)}$$

and

$$C_{max}\text{: } \theta \le 0.6 \qquad \text{(Eq. 12)}$$

A comparative bioavailability study with 12 subjects is conducted in a two-period crossover design. The design information and observed data [AUC, C_{max}, and $\ln(C_{max})$] are shown in Table I, together with the formulation mean values. The analyses of variance used to estimate the experimental errors are given in Tables II and III for AUC and $\ln(C_{max})$, respectively. The validity of the assumed model (Eq. 2) may be verified by examination of the ANOVA residuals. Interested readers will find appropriate procedures in Belsley *et al.* (7).

Bioequivalence Assessment with Respect to AUC—The estimated ratio, given by Eq. 6 is $\hat{\theta} = 90.10/98.35 = 0.916$. The estimated experimental error from the ANOVA (Table II) is 690.4, so that the estimated coefficient of variation (CV) given by Eq. 7 is $\sqrt{690.4}/98.35 = 0.267$. The posterior probability that θ lies in the interval 0.8–1.2 (Eq. 11) may then be calculated from Eqs. 3–5 to give $Pr(0.8 \le \theta \le 1.2) = 0.846$. The cumulative posterior distribution function is shown in Fig. 3; this may also be used, as illustrated, to calculate the probability of the bioequivalence condition.

Bioequivalence Assessment with Respect to C_{max}—The estimated ratio for log-normally distributed data (3) is given by:

$$\hat{\theta} = \exp[\overline{\ln(C_{max})_{NEW}} - \overline{\ln(C_{max})_{STD}}]$$
$$= \exp[4.658 - 5.391] = 0.481 \qquad \text{(Eq. 13)}$$

The estimated error standard deviation from the ANOVA (Table III) is $S = \sqrt{0.147} = 0.383$. The posterior probability that $\theta \le 0.6$ (Eq. 12) may then be calculated from Eqs. 3, 9, and 10 to give $Pr(\theta \le 0.6) = 0.906$. The cumulative posterior distribution function for θ is shown in Fig. 4.

DISCUSSION

Analysis of the example presented shows the main advantages of a Bayesian approach over previous approaches:

1. Earlier methods for bioequivalence assessment consisted of testing the null hypothesis of no difference between formulations. In the present example this null hypothesis would be rejected for C_{max} since the F-ratio in the ANOVA (Table III) is large, but not for AUC, since the F-ratio in the ANOVA (Table II) is small.
2. The assessment of the bioequivalence condition with respect to AUC for the specified interval 0.8–1.2 (Eq. 11) yielded a posterior probability of 0.846. There is, therefore, not enough evidence for claiming bioequivalence. However, the calculation of the cumulative distribution function allows other aspects of interest to be investigated. Thus it may

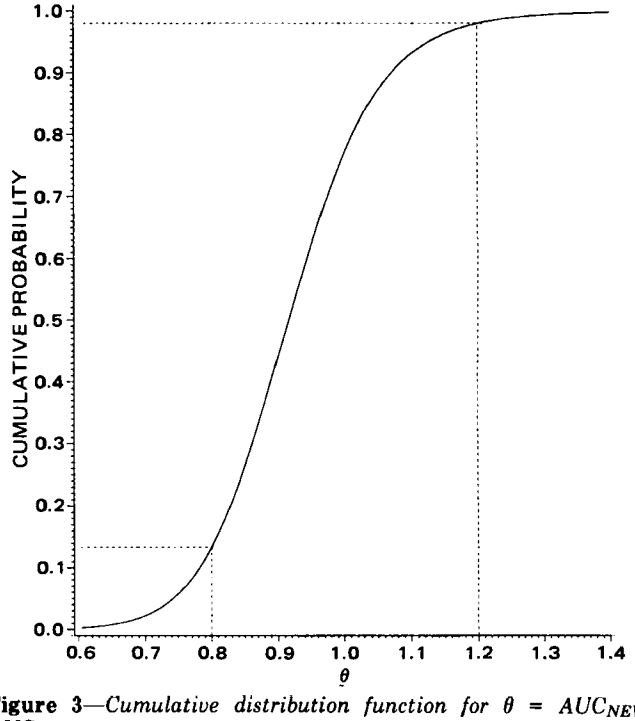

Figure 3—*Cumulative distribution function for* $\theta = AUC_{NEW}/AUC_{STD}$.

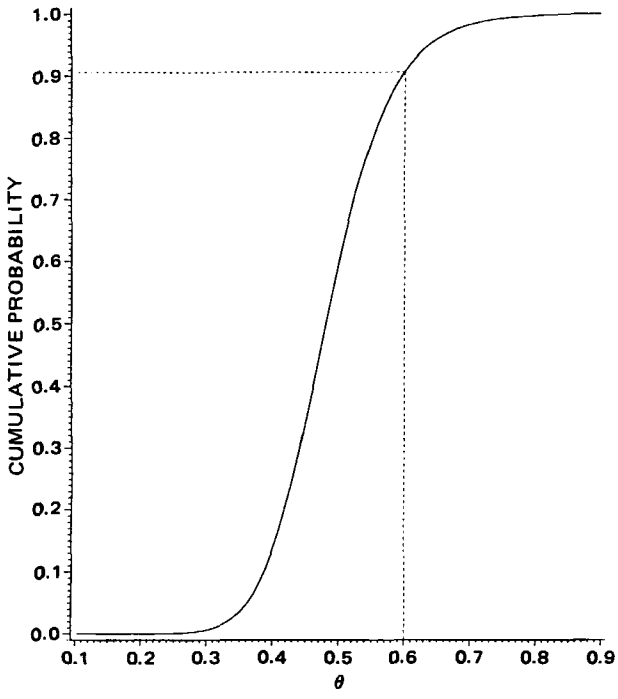

Figure 4—*Cumulative distribution function for* $\theta = (C_{max})_{NEW}/(C_{max})_{STD}$.

be of interest to know the probability that θ is >0.8. This may be read from Fig. 3 and is 0.866—a probability which still might not be high enough to state bioequivalence. In addition it might be desirable to know the probability of θ being >0.7, which also may be read from Fig. 3 and is 0.98.

3. The assessment with respect to C_{max}, that θ is <0.6 (Eq. 12) yielded a posterior probability of 0.906. Similarly it might be desirable to know the probability of θ being >0.7. This probability can be read from Fig. 4 and is 0.02.

4. The method of symmetrical confidence limits proposed by Westlake (1) causes the loss of information since it gives the false impression that the ratio is symmetric about 1. As Mandallez and Mau (3) have shown, the symmetric confidence interval approach may give exactly the same 95% confidence intervals for two sets of data while having completely different posterior distributions for θ, because of the differing variances and locations of the posterior distributions.

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In Vivo and *In Vitro* Release of Macromolecules from Polymeric Drug Delivery Systems

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Received January 26, 1982, from the **Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139* and ‡*Department of Surgery, Children's Hospital Medical Center, Boston, MA 02115.* Accepted for publication August 30, 1982.

Abstract □ *In vivo* release rates of a macromolecule from an ethylene–vinyl acetate copolymer have been shown to be indistinguishable from those of identical implants tested *in vitro*. The studies were conducted for ~2 months, and two different techniques were used to assess release rates. One of these techniques, using [^{3}H]inulin as a marker, may be particularly useful in future studies assessing *in vivo* release rates from drug delivery systems. The appearance of [^{3}H]inulin in the urine of rats bearing implants allowed continuous monitoring of release. A histological evaluation of tissue sections surrounding polymer implanted for 7 months showed no inflammatory cell reaction.

Keyphrases □ Drug delivery systems—ethylene–vinyl acetate copolymer matrix, inulin, release kinetics, *in vitro–in vivo* comparison □ Ethylene–vinyl acetate copolymer—sustained release of inulin, release kinetics, *in vitro–in vivo* comparison □ Inulin—sustained release using polymeric matrices, release kinetics, *in vitro–in vivo* comparison

Since the first report that biocompatible polymers such as ethylene–vinyl acetate copolymer could be used for the controlled release of macromolecules (mol. wt. > 1000) (1), these systems have been used by different investigators in biological (2–11), ophthalmological (12–17), neurological (18, 19),and microbiological research (20, 21). Macromolecules such as enzymes (22), antigens (23), and insulin (24) have been released in biologically active form for up to 6 months *in vivo*. Extensive studies *in vitro* have demonstrated that the release rates of drugs from these devices can be adjusted over a 2000-fold range by simple alterations in the fabrication procedures of the macromolecule–polymer matrices (25).

The macromolecules incorporated into these polymer matrices are usually proteins. Thus, once released *in vivo*, they are degraded to amino acids and recycled to other body proteins. Neither the native proteins nor their metabolites are excreted. For this reason, it has been difficult to directly measure the absolute release rates of such macromolecules *in vivo*. We now report two new methods to measure *in vivo* release which demonstrate that release kinetics from ethylene–vinyl acetate copolymer implants *in vivo* are indistinguishable from identical implants tested *in vitro*. In one method, release rates of ^{14}C-labeled proteins were determined by assaying the remaining radiolabeled protein in the implants at various time points *in vivo* and *in vitro*. In the second method, the use of the polysaccharide inulin, which is totally excreted (26, 27), permitted direct *in vivo* monitoring of release kinetics by collecting urine and assaying for inulin. These studies should enable investigators to employ ethylene–vinyl acetate copolymer matrices with the knowledge that predetermined *in vitro* release kinetics will be followed *in vivo*.

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