

Sơ lược về thử nghiệm sự an-định của dược-phẩm

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Tóm lược

Tính an-định của một dược-phẩm, nhất là những thuốc mới thuộc loại sinh-kỹ-thuật, là một vấn đề vô cùng quan trọng. Những thử nghiệm về sự an-định cho ta biết phẩm-chất của thuốc trong thời gian còn hiệu-lực, nghĩa là lúc thuốc còn dùng được. Nếu thời gian này quá ngắn, thì việc xử dụng trở thành khó khăn và tốn kém, vì sự bào-chế, kiểm-định, tồn trữ, phân phối thuốc tới tay bệnh nhân thường phải mất rất nhiều thời giờ. Dược-phẩm khi bị thoái hóa hay hư hao trong khi tồn trữ không được sinh ra những chất phụ hay phó sản độc hại cho người tiêu thụ. Mỗi dược phẩm thường ở một trường hợp cá biệt. Hoạt-chất của dược-phẩm có thể là một hóa-chất, một chất sinh-học, hay một chất trắc-nghiệm. Khi hoạt-chất còn lại ít hơn 90% phân lượng đã khai báo và ghi trên nhãn, thì thuốc coi như đã đáo hạn và hết hiệu-lực. Tìm kiếm được một công-thức để an-định dược-phẩm, nghĩa là làm tăng thời gian hiệu-lực, là một nhiệm vụ chung của các khoa học gia trong ban khảo-cứu/phát-triển các phương-thức bào-chế. Bài này tóm lược đại cương những nguyên-lý, cách thực hành, cùng một số chỉ dẫn của cơ quan quản-trị Thực Dược Phẩm Hoa Kỳ về thử nghiệm xác định thời gian hiệu-lực. Cách tính thời gian hiệu-lực của một thử nghiệm thời-thực được trình bày ở phụ-lục 1. Thí dụ về một thử nghiệm gia-tốc dùng nhiệt độ cho một kháng-thể đơn bản được trình bày ở phụ-lục 2. Một vài tài liệu và địa chỉ những mạng lưới toàn cầu liên hệ đến vấn đề này được ghi trong thư mục tham khảo.

Thuật ngữ

Tính an-định:
Chất sinh học:

Stability
Biologic

| | |
|----------------------------------|--------------------------|
| Thuốc loại sinh-kỹ-thuật: | Biotech product |
| Kháng-thể đơn bản: | Monoclonal antibody |
| Thời gian hiệu-lực: | Expiration dating period |
| Hoạt-chất: | Active substance |
| Phương-thức bào-chế: | Formulation |
| Cơ quan quản-trị Thực Dược Phẩm: | FDA |
| Trắc-nghiệm: | Test |
| Thử nghiệm: | Testing |
| Thử nghiệm thời-thực: | Real-time testing |
| Thử nghiệm gia-tốc: | Accelerated testing |
| Năng lượng kích động: | Activation energy |
| Phản ứng bậc không: | Zero order reaction |
| Phản ứng bậc một: | First order reaction |
| Phản ứng bậc hai: | Second order reaction |
| Kiểu mẫu động học: | Kinetic models |
| Hằng số vận tốc: | Rate Constant |

An Overview for Drug Stability Testing

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Summary

In the last step of the product/process development, the main objectives of the Formulation group are optimizing the drug delivery system and increasing the stability of a drug product. When the process is validated, then the testing of drug stability in the Manufacturing group will insure that the medicine retain its identity, strength and quality in the market throughout the period up to the expiration date.

The knowledge in many areas of the drug such as potency, metabolism, and physical/chemical/biochemical pathways allows the rational development of analytical methods for the expiration dating.

In this overview, some principles, practices and stability testing guidelines from the FDA of the US are briefly presented.

I. DRUG STABILITY

It is note that one could find in literature various examples of drug formulation using trehalose and hydrophobic sugar glasses (1), liposomes (2, 12), cyclodextrins (3, 19), of chemical kinetics and drug stability (4, 5, 6, 7), and of expiration dating and shelf life estimation (8, 9, 10). Also, one can get considerable amounts of updated information in the web sites of FDA including the FDA modernization act of 1997 (FDAMA) (13, 14, 15) and in that of the European Agency for the Evaluation of Medicinal products EMEA (16). In addition, one can have relevant services as provided by many independent labs (17, 18, 19, 20).

1. Definition:

The stability means compliance of the drug product within the specifications. The stability testing insures the quality of the drug product as defined by its content of active ingredient, its purity and its organoleptic, physicochemical and biological properties. The drug could be a chemical, a biologic or a medical device. In general, biologics depend mostly on optimum formulation and good storage conditions for stability.

2. *Measurement:*

The methods used to assess and control stability are based on rate constants of degradation reactions of the drug.

The shelf life of the drug can be calculated if the rate of loss "k" of the drug with time at storage temperature is known.

If $[A]_0$ and $[A]_t$ are the initial active concentration and the residual active concentration at time t, the following rate equations describe the usual models:

| | | | |
|---------------|--------------------|---|-------------------------|
| zero order: | $[A]_t$ | = | $[A]_0 - kt$ |
| first order: | $\text{Ln } [A]_t$ | = | $\text{Ln } [A]_0 - kt$ |
| second order: | $1/[A]_t$ | = | $1/[A]_0 + kt$ |

The drug product expiration dates are usually based on assumed zero- or first-order kinetics. The shelf-life $t_{0.90}$ is the time at which decomposition reaches 10% or activity decreases to 90%. The time should be determined at which the 95% one-side lower confidence limit for the mean degradation curve intersects the lower acceptable specification limit (appendix 1). This will assure that the average drug characteristics of the batch are within specifications up to the end of the expiration period.

II. STABILITY PROTOCOL

1. *General Product Information:*

The basic information of the drug must be presented:

- Name.
- Dosage form.
- Strength.
- Formulation.
- Labeling.
- Container-closure: composition, type and size.

2. *Specifications and Test Methodology Information:*

The specifications on physical, chemical, biological and microbiological characteristics of the drug must be described. A definition of potency is usually needed for biological activity of a drug.

The analytical methodology should be validated and presented with method accuracy, precision and suitability (11).

The method measuring the trace amount of harmful by-product or unwanted degraded product during drug storage should be known with acceptable limit of detection.

3. Study Design and Study Conditions:

The sampling plan, number of units and sampling times are selected according to statistical quality control methods.

The testing of drug products for reconstitution at the time of dispensing (as directed on the labeling) must be defined. The same requirement is needed after they are reconstituted.

The duration of the study and storage conditions: temperature, humidity and light should be specified. For example, the Human Medicines Evaluation Unit of the EMEA defined significant change as failure to meet the specifications with long term testing, at temperature $25^{\circ} \pm 2^{\circ} \text{C}$ and relative humidity $60\% \pm 5\% \text{RH}$ for 12 months, and with accelerated testing at $40^{\circ} \pm 2^{\circ} \text{C}$ and $75\% \pm 5\% \text{RH}$ for 6 months.

4. Stability Data/Information:

The lot number from research, pilot or production must be provided with the corresponding manufacturing date. The age of the bulk/active substance used in the testing should be mentioned. The analytical data and source of data points must be defined.

All relevant information of previous formulations or container-closure systems should be provided.

5. Data Analysis and Conclusions:

The appropriate statistical methods and formulae used in the analysis must be documented. The calculations, statistical analysis and graphs to evaluate data should be provided. The results of statistical tests for potency estimates as well as the proposed expiration date and its justification must be presented.

The release specifications are defined to warrant an acceptable minimum

potency at the initial release for full expiration dating period.

III. STABILITY STUDY

1. Real-time stability:

This stability study at storage condition is the most reliable, but unfortunately it takes a long time and is very costly in the development phase of the drug.

2. Accelerated stability:

The accelerated stability study predicts the expiry date using exaggerated storage conditions. The drug substance can be stressed as many ways as possible, using temperature, humidity, light, pH, solvents, buffers etc...

The rate constant k is observed to have an exponential dependence on temperature. Where k is the reaction rate constant of any order, A and E_a are constants, and T is the absolute temperature, according to Arrhenius:

$$k = A \exp(-E_a/RT)$$

The activation energy $-E_a$ can be calculated as equal to $R \cdot \text{Slope}$ with $R = \text{Gas Constant} = 1.987 \text{ cal K}^{-1} \text{ mol}^{-1}$.

The activation energy E_a is an energy barrier of the system that the reactants must pass before becoming products. The usual range of E_a is about 12 to 24 kcal/mol, with hydrolysis: 14-20 kcal/mol and oxidation: 23 kcal/mol

The E_a depends on formulation, for example: the phenylbutazone in water has three rates: k_1, k_2, k_3 of oxidation/hydrolysis corresponding respectively to E_{a1}, E_{a2}, E_{a3} of 24.4, 26.7, 36.2 kcal/mol. In solvent such as dimethyl formamide, diethyl carbinol, propylene glycol, the E_a 's are lower, from 3.9 to 7.7 kcal/mol, giving a shorter shelf life, only from 18 days to 113 days.

The Q_{dT} factor, ratio of reaction rates at two temperatures differing by dT degrees, can be calculated as:

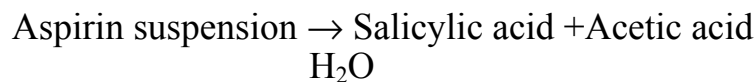
$$Q_{dT} = \frac{k_{T+dT}}{k_T} = \exp \left\{ \frac{-E_a}{R} \left(\frac{1}{T+dT} - \frac{1}{T} \right) \right\}$$

This factor is used to predict shelf life at 4°C, knowing shelf life at higher temperature. Usually, Q_{10} from 20° to 30° C equals to 2, 3 and 4 with E_a 's from 12.2 to 24.5 kcal/mol. This means that the rate increases 6 to 32 times at 25°C from initial rate at 0° C.

IV. EXAMPLES

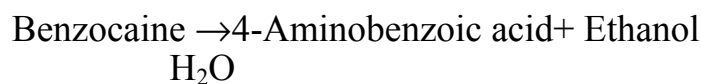
The rate and order of reactions of some drugs are presented with kinetic models:

1. *Zero order:* $[A]_t = [A]_o - kt$



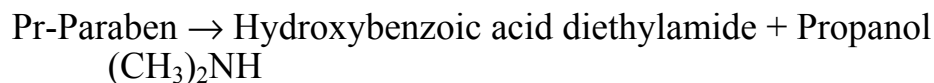
$k = 0.0075 \text{ mol l}^{-1} \text{d}^{-1}$ or $1.5\% \text{ d}^{-1}$
 $t_{0.90} = 6.67 \text{ days}$

2. *First order:* $\ln [A]_t = \ln [A]_o - kt$



$k = 0.05 \text{ w}^{-1}$
 $t_{0.50} = 0.693/k = 13.86 \text{ weeks}$

3. *Second order:* $1/[A]_t = 1/[A]_o + kt$



$k = 0.012 \text{ mmol l}^{-1} \text{w}^{-1}$
 $t_{0.90} = 0.926 \text{ weeks}$

4. *Other kinetic models:*

First order reversible kinetics

Photochemical Isomerisation [A \leftrightarrow B]

Chlorprothixene

Competitive first order degradation

Hydrolysis/Rearrangement [C \leftarrow A \rightarrow B]

O-Acetyl Propanolol

Sequential first order kinetics

Rearrangement/Hydrolysis [A \leftarrow B \rightarrow C]

Betamethasone 17-Valerate

5. *Analysis of a Monoclonal Antibody MoAb degradation study:*

An example of accelerated testing using temperature to predict stability of a monoclonal antibody MoAb against carcino-embryonic antigen CEA is described. Samples are stressed at 37°C and the percent immunoreactivity % IR are recorded as follows:

Test samples:

MoAb in 140mM Phosphate, pH 8.5, 37°C

| <u>Sample ID</u> | <u>% IR</u> |
|------------------|-------------|
| 0 week | 95.8 |
| 1 week | 89.8 |
| 2 weeks | 88.4 |
| 3 weeks | 75.8 |
| 4 weeks | 75.6 |
| 5 weeks | 66.3 |
| 6 weeks | 62.6 |
| 7 weeks | 58.5 |
| 8 weeks | 51.7 |

Kinetic model:

Different models are tested and results are tabulated in the following paragraph:

| <u>MODEL</u> | <u>R</u> | <u>[IR%]₀</u> | <u>SS</u> |
|-----------------------|-----------|--------------------------|-----------|
| Zero order | 0.9907989 | 95.9 | 34.1 |
| 1 st order | 0.9897601 | 98.2 | 44.1 |
| 2 nd order | 0.9812260 | 102.3 | 100.1 |

Conclusion:

The best model describing the degradation of this monoclonal antibody is the zero order kinetics. It gives the highest correlation coefficient R of 0.9907989, the closest estimated initial %IR of 95.9 and the smallest sum of squares SS of 34.1.

The percent immunoreactivity of this monoclonal antibody replaces its potency in the calculation of loss of activity with time. The antibody vials at up or inverted positions of storage are used to detect effect of stoppers, but no difference has been found. The testing temperatures are 22°C, 37°C and 45°C. All physical, chemical, biological and microbiological characteristics of this antibody are tested at time intervals, but only results for activation energy, rate constants and shelf-lives at various temperatures calculated using Excel program are presented in appendix 2.

6. *Some calculation programs used in the Stability Study:*
Any of the following software could be used: RS/1 from BBN, Statistical Analysis System SAS, Excel PC, Q-basic or Irwin's computer solutions.

Conclusion

Some aspects and examples of the drug stability testing are presented. The accelerated testing to predict expiry dating of a monoclonal antibody against CEA is described. In a dosage form, each drug substance represents a particular case and should be treated accordingly. A good formulation could only be obtained by a team effort of many scientists in the product/process development phase.

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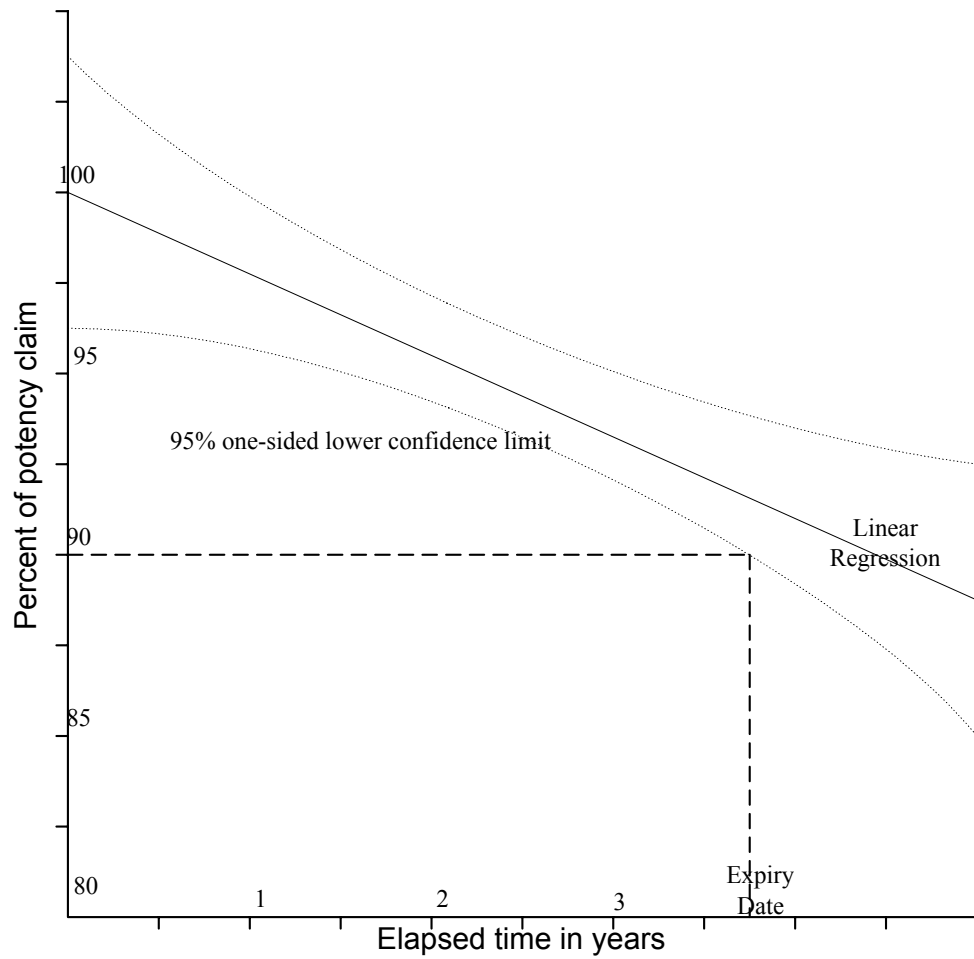
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Appendix 1
The lower bound method: If the specification
limit is 90%, an expiration dating period of
3.7 years would be granted



Appendix 2:
Accelerated Stability Testing for a MoAb

ARRHENIUS ANALYSIS

Initial %IR = 94.8

Ea = 19.34 Kcal/mol = 80.92 KJ/mol

| AGE (week) | TEMP (C) | U/I %IR | 1/T | Ln k | Trend | |
|---------------|----------|---------|------|---------|-----------|-----------|
| 2 | 22 | U | 92.7 | 0.00339 | -4.556380 | -5.548497 |
| 2 | 22 | I | 94.7 | 0.00339 | -7.600902 | -5.548497 |
| 2 | 37 | U | 93.4 | 0.00322 | -4.961845 | -3.953520 |
| 2 | 37 | I | 92.6 | 0.00322 | -4.509860 | -3.953520 |
| 2 | 45 | U | 89.5 | 0.00314 | -3.630611 | -3.164362 |
| 2 | 45 | I | 83.5 | 0.00314 | -2.873515 | -3.164362 |
| 4 | 22 | U | 86.5 | 0.00339 | -3.875209 | -5.548497 |
| 4 | 22 | I | 94.3 | 0.00339 | -6.684612 | -5.548497 |
| 4 | 37 | U | 87.1 | 0.00322 | -3.950244 | -3.953520 |
| 4 | 37 | I | 85.2 | 0.00322 | -3.729701 | -3.953520 |
| 4 | 45 | U | 75.3 | 0.00314 | -3.021050 | -3.164362 |
| 4 | 45 | I | 77.8 | 0.00314 | -3.158251 | -3.164362 |
| 6 | 22 | U | 93.2 | 0.00339 | -5.926926 | -5.548497 |
| 6 | 22 | I | 92.8 | 0.00339 | -5.703782 | -5.548497 |
| 6 | 37 | U | 81.8 | 0.00322 | -3.831980 | -3.953520 |
| 6 | 37 | I | 82.9 | 0.00322 | -3.920391 | -3.953520 |
| 6 | 45 | U | 64.0 | 0.00314 | -2.969415 | -3.164362 |
| 6 | 45 | I | 67.5 | 0.00314 | -3.090043 | -3.164362 |
| 8 | 22 | U | 91.1 | 0.00339 | -5.376279 | -5.548497 |
| 8 | 22 | I | 90.2 | 0.00339 | -5.158555 | -5.548497 |
| 8 | 37 | U | 75.9 | 0.00322 | -3.745450 | -3.953520 |
| 8 | 37 | I | 77.7 | 0.00322 | -3.845533 | -3.953520 |
| 8 | 45 | U | 58.1 | 0.00314 | -3.081835 | -3.164362 |
| 8 | 45 | I | 61.1 | 0.00314 | -3.167114 | -3.164362 |
| 12 | 22 | U | 87.8 | 0.00339 | -5.144167 | -5.548497 |
| 12 | 22 | I | 88.8 | 0.00339 | -5.298317 | -5.548497 |
| 12 | 37 | U | 67.3 | 0.00322 | -3.775891 | -3.953520 |
| 12 | 37 | I | 66.5 | 0.00322 | -3.747215 | -3.953520 |

Calculations

Ln k = Ln A - Ea/RT

Shelf life at 4 °C

Temperature C = 4

T = 277.15

Ln k = -7.690365

k = 0.000457

t_{0.9} (week) = **230.4**

Shelf life at 25 °C

Temperature C = 25

T = 298.15

Ln k = -5.216663

k = 0.005425

t_{0.9} (week) = **19.4**

Shelf life at 37 °C

Temperature C = 37

T = 310.15

Ln k = -3.953520

k = 0.019187

t_{0.9} (week) = **5.5**

N.B.: U/I = up or inverted storage position
k = absolute value
t_{0.9} = lower bound method shelf life