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Review

A Critical Appraisal of Drug Stability Testing Methods

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The determination of potency or shelf life, impurity limit testing, and study of reaction mechanisms are considered as different aspects of drug stability. These aspects traditionally have been treated in isolation. The current criterion for a stability-indicating assay is criticized and the merits of choosing reactant or decomposition product for monitoring decomposition are discussed. The initial-rate method of determining reaction order and rate constants by analysis of decomposition product is described and its potential advantages over traditional integral methods are discussed. Examples of the application of the initial-rate method to simple and complex drug decomposition systems for the determination of decomposition rate constants are given. Applications to limit testing and study of reaction mechanisms are outlined and the dependence of the initial-rate method and decomposition product analysis on modern chromatographic methods is emphasized.

KEY WORDS: stability; initial-rate method; decomposition product; stability-indicating assay.

Determining the stability of a drug often means determining the time over which a drug or drug product will maintain an arbitrary proportion of its potency. Direct measurement of potency by assay of the intact drug over an extended time scale is the most unequivocal method of determining the shelf life.

The procedure is time-consuming and places considerable demands upon the long-term precision of the analytical methods used. A major step forward resulted from the application of general chemical kinetic ideas to stability testing of drugs (1). This allowed the application of order of reaction concepts and, via temperature stressing, enabled the Arrhenius equation to be used. Thus, rate constants and shelf lives could be predicted at storage temperatures based on relatively short time studies carried out at elevated temperatures. Such isothermal stability testing apparently provided additional information in terms of activation energies of drug decompositions which is of value in elucidating the mechanism of such reactions.

The temperature dependence of drug decompositions was further utilized in the technique of nonisothermal stability testing in which, by increasing the temperature during the course of a drug decomposition, the quantities of rate constant and activation energy may be obtained in a single kinetic experiment (2). Both isothermal and nonisothermal methods of stability determination are based upon well-established physical–chemical principles but both contain assumptions which may not be acceptable in the context of the complex decomposition patterns of many drugs.

Stability also means the production of toxic or unaesthetic impurities which limit the shelf life of the drug even when adequate potency is maintained. This aspect is quite separate in many workers' minds from the quantitative determination of decomposition rates and is dealt with by the imposition of arbitrary limit tests. These specify maximum levels of such impurities acceptable for pharmacopoeial standards to be maintained. Others may consider that the purpose of studying the stability of a drug is primarily to determine its mechanism of decomposition, possibly with a view to obtaining information on the physical and chemical factors that cause instability, control of which may stabilize the drug or drug formulation and lead to increased shelf life or improved safety.

These three aspects of drug stability have been kept separate and treated as unrelated investigations. This may have been as a result of the widely different analytical methodology used in the various investigations. In mechanism studies the nature of the decomposition products and intermediates is the main requirement. In impurity limit testing, semiquantitative analytical methods have usually been employed to monitor levels of decomposition products. Such tests have required a high specificity and sensitivity to locate low levels of impurity in the presence of intact drug. Shelf-life determinations, however, require quantitative assay of drug or drug decomposition product.

The importance of analytical methodology in drug decomposition has been extensively reviewed (3). The classical spectroscopic methods have been augmented by highly specific chromatographic techniques, such as high-performance liquid chromatography (hplc). The coining of the term "stability-indicating assay," taken to mean "a procedure which affords specific determination of a drug substance in the presence of its decomposition products," indicates the importance of analytical method in the study of drug decompositions (4).

This definition of a stability-indicating assay, while an advance on nonspecific assay techniques which can result in unreliable measurements of drug stability (5), demonstrates the limited view that is currently held with regard to drug stability investigations. It assumes that the species that should be assayed is the undecomposed drug. While this may be the obvious choice when considering shelf life, it yields no information on the nature, number, or amount of degradation products formed, and this information is of con-

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siderable interest in both the toxic limit testing and the mechanism aspects of stability.

Choice of the undecomposed drug as the species for quantitative stability determination suffers disadvantages, particularly when dealing with relatively stable drugs. The precision of the method must be high if the extent of decomposition measured is small. This is typified by the stability study of azathioprine, where less than 7% decomposition was measured after 5 hr at 85°C (6). In such situations the calculation of rate constants is very approximate. In addition, the determination of the order of reaction is impossible under these conditions. For the determination of reaction order and subsequent calculation of rate constants, extents of decomposition in the region of 75% should be followed (7). It is also impractical to distinguish among zero-, first-, and second-order behavior at extents of decomposition below 25% (8) by the use of integrated rate equations. This produces the anomalous situation that, in order to measure the t_{90} value from decomposition rate constants, it is necessary to decompose the drug by 75%.

The restrictions indicated above have resulted in the extensive use of temperature stressing. This use of high temperatures removes the stability determination from the practical situation. The shelf life determined in this way relies upon the validity of the extrapolation to storage temperature. Reports have been published regarding the statistical validity of such extrapolations (9) and nonlinear Arrhenius plots have been obtained (10). Nonisothermal methods assume the validity of the Arrhenius equation and require arbitrary choice of temperature variables such as heating rate.

There has been some controversy in the stability literature as to whether parent drug or a decomposition product should be assayed for the purposes of determining stability (11). The preferred species is the undecomposed drug. Where a decomposition product has been measured this has often been as a result of analytical expediency. Examples of this are dexoxadrol (12) and hydrochlorothiazide (13). In these instances the stoichiometry of the reaction has been used to obtain the concentration of parent drug remaining.

Recently decomposition reactions have been studied by following decomposition product appearance for the reason that this allowed more sensitive detection of decomposition. That is, decomposition product could be observed to increase, while the inherent imprecision of the method could not reliably determine a decrease in the original drug. Examples of these investigations include morphine (14), noxythiolin (15), and several investigations on the decomposition of D-glucose in infusion fluids (16–18). Such product measurement is now, with the advent of hplc, more feasible than hitherto, and the conversion to intact drug concentration is not required in order to establish the rate of a given drug decomposition. When product rather than reactant is measured, the differential methods of classical chemical kinetics are more appropriate than the traditional integrated rate equations. For a simple drug decomposition reaction represented as

$$D \rightarrow P$$

where D represents the undecomposed drug and P the single decomposition product, the decomposition rate constant k is usually determined by fitting drug concentration–time data to one of the integrated rate equations.

$$\begin{aligned} [D]_t &= [D]_0 - k_0 t, && \text{zero order} \\ \ln[D]_t &= \ln[D]_0 - k_1 t, && \text{first order} \\ 1/[D]_t &= 1/[D]_0 + k_2 t, && \text{second order} \end{aligned}$$

These equations are all cast in terms of the concentration of reactant. The order is decided by the fit of the experimental data to the appropriate equation and rate constants are calculated accordingly.

The basis of the above relationships lies in the differential equations defining the order of reaction, i.e.,

$$\begin{aligned} -d[D]/dt = d[P]/dt &= k_0[D]^0 \\ &= k_1[D] \\ &= k_2[D]^2 \end{aligned}$$

These equations show that the rate of the reaction can be determined by measuring either D or P. If the reaction is followed for only a small extent the term $[D]$ will be essentially constant. Thus, under these conditions, any such decomposition will show zero-order kinetics. The order of a reaction is dependent upon the concentration range over which the reaction is studied and reaction orders with respect to time and concentration have been distinguished (19). Reaction order is essentially an experimental quantity, unlike molecularity. Thus a zero-order rate constant may be calculated for a drug decomposition more rapidly by monitoring $[P]$ than $[D]$ in the above equation. This can be appreciated when it is realized that it is possible to measure an increase of 1% in P more precisely than to measure a decrease of 1% in D directly, particularly when highly specific methods are used. This has been established using aspirin as a model drug system (20).

Most drug decompositions are first order when studied over large extents of reaction. Conventional first- and second-order rate constants can, however, be related to this apparent zero-order rate constant (21). It has been shown that over limited extents of reaction

$$d[P]/dt = k_0 = k_1[D]_0 = k_2[D]_0^2$$

Thus, measurement of the initial reaction rate in terms of the product together with a knowledge of the initial molar concentration of the reactant allows conventional rate constants to be calculated using this initial rate method.

The order of a reaction can be difficult to determine and the validity of assuming first or zero order has been discussed (22). The initial-rate method allows the reaction order to be established unequivocally by rearranging the general equation.

$$d[P]/dt = k_n[D]^n$$

when n is the order of reaction to the form

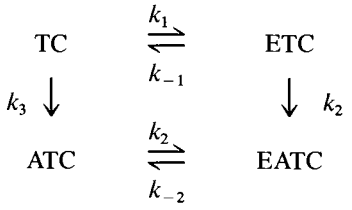
$$\log d[P]/dt = \log k_n + n \log [D]$$

By measuring $d[P]/dt$ over small extents of reaction for several values of $[D]$, the value of n may be determined. This has been employed in the reaction producing 5-hydroxymethylfurfural from D-glucose (17).

The advantages of the initial-rate method are obvious when considering decompositions producing a single well-

characterized decomposition product and could find useful application in assessing the stability of different formulations or packaging of a given drug. What is not so obvious is its utility in considering complex drug decompositions where either consecutive or parallel decompositions occur.

The decomposition of tetracycline (TC) in acid solution represents such a drug decomposition where reversible equilibria are also involved. Tetracycline undergoes reversible epimerization to epitetracycline (ETC) and also dehydration to anhydrotetracycline (ATC). ATC reversibly epimerizes to epianhydrotetracycline (EATC), which can also be formed from the dehydration of ETC.



This system has been studied by completely decomposing TC under strongly acid conditions and monitoring reactant and products. By iterative curve fitting, values were obtained for the individual rate constants (23). More recently (24) this reaction was studied using the initial-rate approach where TC, ETC, and ATC were used successively as reactants. By confining the extent of each decomposition to extents of reaction less than 5%, a maximum of two products was separated and quantitated. This allowed determination of all rate constants other than k_{-1}.

The temperature dependence of each reaction was determined separately and log k–pH profiles were established in a reasonable time scale. The individual activation energies for each reaction were also measured. Study of the product concentration thus provides additional information to that obtained by study of the reactant concentration only.

The initial-rate method requires that the absolute concentration of the species involved must be determined. Thus it requires that the reaction pathway be known and that standard samples of decomposition products are available. This is in contrast to first-order drug decompositions, where any property linearly related to the concentration of reactant may be measured. The initial-rate method has, however, been extended to the study of a potential drug compound, nafimidone [1-(2-naphthoylmethyl)imidazole hydrochloride] (N), which decomposed to a single decomposition product (P) as yet unidentified (25). The concentration of P was estimated by decomposing N to a large enough extent to obtain a measurable decrease in N and assuming that the chromatographic peak observed for P represented the stoichiometric amount corresponding to the decrease in N. Rate constants for the reaction were obtained together with activation energy and pH–log k profiles. It is not yet seen how this procedure may be applied to situations where a drug decomposes to several unknown decomposition products. In such instances it may be possible to simplify the system by control of conditions. The decomposition of mitomycin C, for example, was shown (26) to produce several different decomposition on acid or base hydrolysis. A recent publication, on the other hand, shows that under strongly alkaline conditions a single product is formed (27). Under these conditions the above procedure may be adopted to follow the rate of decomposition. Where a given drug decomposes into several well-characterized species such as the tetracycline situation, the real limitation to the initial-rate method is the feasibility of low-concentration multicomponent analysis.

The initial-rate method using decomposition product measurement has the additional advantage of providing directly information on impurity levels arising from degradation. In the case of tetracycline it is likely that the shelf life is determined by the maximum levels of the toxic EATC produced during decomposition. This is also shown by the limit testing of autoclaved dextrose infusion fluids, where a nonspecific spectrophotometric limit test is applied to control the amount of 5-hydroxymethylfurfuraldehyde (5HMF) and related products. The reaction of dextrose to 5HMF has been shown to be first order with respect to dextrose (19). The use of hplc shows that further oxidation of 5HMF occurs to produce a mixture of furan acids (28). Thus the initial-rate method yields information on different products produced during decomposition. On the basis of these studies a modified limit test has been proposed for such infusion fluids (29).

For a drug assay to be considered truly stability indicating, as well as being capable of determining undecomposed drug in the presence of its degradation products, it should also be capable of quantitating the individual product(s) of possibly different reaction pathways. This will allow the application of the classical method to monitor overall loss of potency and will allow the advantages of the initial-rate method to be realized. Such stringent demands upon an analytical method can be met only by hplc. In recent years there have been considerable advances made in the field of hplc, not only in the control of retention but also in the control of selectivity. That is, the order of retention can often be varied so that such drug decomposition products can be eluted before undecomposed drug. This allows detection of these minor components with maximum sensitivity.

In conclusion, the existing methods of stability determination appear not to relate the various fields of shelf-life determination, limit testing, and study of decomposition mechanism. The initial-rate method, while relatively little used, is becoming increasingly utilized without full appreciation of the kinetic advantages that can be achieved. A change in emphasis in the analytical approach to stability is appropriate at this time and indeed is only now possible because of the development of suitable analytical methodology. It is hoped that future improvements in analytical methods and the application of appropriate kinetic treatment of stability data will produce a more comprehensive approach to drug stability testing.

REFERENCES

1. E. R. Garrett. *J. Pharm. Sci.* 51:811–833 (1962).
2. A. R. Rogers. *J. Pharm. Pharmacol.* 15:101–105T (1963).
3. L. Chafetz. *J. Pharm. Sci.* 60:335–345 (1971).
4. J. A. Mollica, S. Ahuja, and J. Cohen. *J. Pharm. Sci.* 67:443–465 (1978).
5. W. I. Lund and T. Waaler. *Acta Chem. Scand.* 22:3085–3088 (1968).

6. A. F. Fell, S. M. Plag, and J. M. Neil. *J. Chromatogr.* 186:691–704 (1979).
7. J. P. R. Tootill. *J. Pharm. Pharmacol.* 13:75–86T (1961).
8. W. Yang. *Drug Dev. Ind. Pharm.* 7:717–738 (1981).
9. E. R. Garrett. *J. Am. Pharm. Assoc. Sci. Ed.* 45:171–178 (1956).
10. F. Matsui, D. L. Robertson, P. La Fontaine, H. Kolasinki, and E. G. Lovering. *J. Pharm. Sci.* 67:646–650 (1978).
11. J. T. Carstensen, J. B. Johnson, D. C. Spera, and M. J. Frank. *J. Pharm. Sci.* 57:23–27 (1968).
12. L. W. Brown and A. A. Forist. *J. Pharm. Sci.* 62:1365–1367 (1973).
13. C. R. Rehn and J. B. Smith. *J. Am. Pharm. Assoc. Sci. Ed.* 49:386–389 (1961).
14. T. Deeks, S. Davis, and S. Nash. *Pharm. J.* 220:405–497 (1983).
15. W. J. Irwin, A. Li Wan Po, and J. S. Stephens. *J. Clin. Hosp. Pharm.* 9:41–51 (1984).
16. K. R. Heimlich and A. N. Martin. *J. Am. Pharm. Assoc. Sci. Ed.* 49:592–597 (1960).
17. R. B. Taylor, B. N. Jappy, and J. M. Neil. *J. Pharm. Pharmacol.* 23:121–129 (1971).
18. R. B. Taylor and Y. C. Sood. *J. Pharm. Pharmacol.* 30:510–511 (1978).
19. G. Pannetier and P. S. Souchay. *Chemical Kinetics,* Elsevier, New York, 1967.
20. A. S. H. Shivji. Ph.D. thesis (1986).
21. J. T. Carstensen and K. S. E. Su. *Bull. Parent. Drug Assoc.* 25:287–302 (1971).
22. N. G. Lordi and M. W. Scott. *J. Pharm. Sci.* 54:531–537 (1965).
23. P. H. Yuen and T. D. Sokoloski. *J. Pharm. Sci.* 66:1648–1650 (1977).
24. R. B. Taylor, D. G. Durham, and A. S. H. Shivji. *Int. J. Pharm.* 26:259–266 (1985).
25. R. B. Taylor, D. G. Durham, A. S. H. Shivji, and R. Reid. *J. Chromatogr.* 353:51–59 (1986).
26. D. Edwards, A. B. Selkirk, and R. B. Taylor. *Int. J. Pharm.* 42:21–26 (1979).
27. J. H. Beijnen, J. Den Hartigh, and W. J. M. Underberg. *J. Pharm. Biomed. Anal.* 3:56–69 (1985).
28. D. G. Durham, C. T. Hung, and R. B. Taylor. *Int. J. Pharm.* 11:31–40 (1982).
29. C. T. Hung, A. B. Selkirk, and R. B. Taylor. *J. Clin. Hosp. Pharm.* 7:17–23 (1982).