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Evaluation of drug stability data by analog-hybrid computer: application to lorazepam

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Summary

The criteria for evaluation of reaction order and pathways of degradation in drug stability studies were investigated. The applicability of classical methods (linear regression, linear regression forced through origin, mean square error) and analog-hybrid simulation is discussed using lorazepam solution stability as an example. Experimental data for lorazepam degradation and quinazolinecarboxaldehyde derivative formation were obtained by HPLC and then evaluated on microcomputer, Apple II, and analog-hybrid computer, EAI 580. First-order kinetics for lorazepam degradation at declared storage conditions was confirmed. In addition the kinetic model for lorazepam degradation pathway was derived. The advantages of analog-hybrid simulation in such studies are pointed out.

Introduction

Drug stability studies concern physicochemical, pharmacotherapeutic and toxicological aspects of the drug itself and/or its dosage form. The precedence and importance of particular aspects depends on the stage of drug development. Time dependence of drug content under different storage conditions represents the main criterion in drug stability evaluation. Therefore kinetics of drug degradation as well as possible mechanisms must be studied. In this connection it is important to determine the order of the degradation reaction, especially when stability tests are

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performed under accelerated storage conditions (Carstensen and Su, 1971).

There are several methods usually used for determination of reaction order such as substitution, half-life, least-squares regression and weighted least-squares regression methods (Carstensen, 1972; Wagner, 1975). Analog-hybrid simulation can also be used for studying degradation kinetics and additionally for degradation pathways ascertainment (Kmetec et al., 1983). Differential equations dealing with several consecutive and/or parallel steps, which are usually involved in chemical processes, are relatively easily solved by analog-hybrid computers in a short period of time (Karba et al., 1978; Karba et al., 1982; Young et al., 1981). The aim of the present work is to justify the use of analog-hybrid simulation and its advantages in stability studies using lorazepam–water solution stability as an example.

Theory

Stability data can be treated using various approaches, when the order of the degradation kinetics has to be determined. Least-squares linear regression is the method which is usually used for determination of the best possible straight line running through the data points. Minimizing the sum of squared differences between the function and experimental points, the regression line described by Eqn. 1 can be derived.

$$y = a_{yt} + b_{yt} \cdot t \tag{1}$$

where y, regarding the order of reaction, represents either the percentage or the natural logarithm of percentage of undegradated drug content; t is time; and a_{yt} and b_{yt} are the regression coefficients calculated using Eqns. 2 and 3.

$$a_{yt} = \bar{y} - b_{yt} \cdot \bar{t} \tag{2}$$

$$b_{yt} = \frac{n\Sigma ty - \Sigma t \Sigma y}{n\Sigma t^2 - (\Sigma t)^2} \tag{3}$$

where $\bar{y}$ and $\bar{t}$ are the arithmetic means of the dependent and independent variables and n is the number of experimental points. It is evident that the coefficient b_{yt} used in Eqns. 1–3 signifies the degradation rate constant of a drug under investigation. The Pearson correlation coefficient (r) measures the linear association between y and t. It is calculated using Eqn. 4.

$$r = \frac{n\Sigma ty - \Sigma t \Sigma y}{\left[\left(n\Sigma t^2 - (\Sigma t)^2\right)\left(n\Sigma y^2 - (\Sigma y)^2\right)\right]^{0.5}} \tag{4}$$

An additional criterion, i.e. MSE (mean square error) (Eqn. 5) must be introduced when comparing linear regression lines determined in linear and semilogarithmic

coordinate systems (Carstensen, 1972; Yang, 1981). The reason for such a decision lies in the fact that some prerequisites concerning least-squares linear regression are not met, when semilogarithmic transformations are involved.

$$MSE = \frac{\Sigma(y - y_c)^2}{n - 2} \tag{5}$$

where y_c represents the percentage of drug content calculated from the regression line.

In drug stability studies it would often be desirable to plot a line of y versus t, which passes through the 100% point (Wagner, 1975). The equation of this line in a linear coordinate system is given by Eqn. 6, in which the symbols used have already been described above. The equation assuming semilogarithmic transformation can easily be derived. It is obvious that also in this case MSE must be considered.

$$y = 100 - b_{y_t} \cdot t = 100 - \left(\frac{\Sigma(100 - y)t}{\Sigma t^2} \right) \cdot t \tag{6}$$

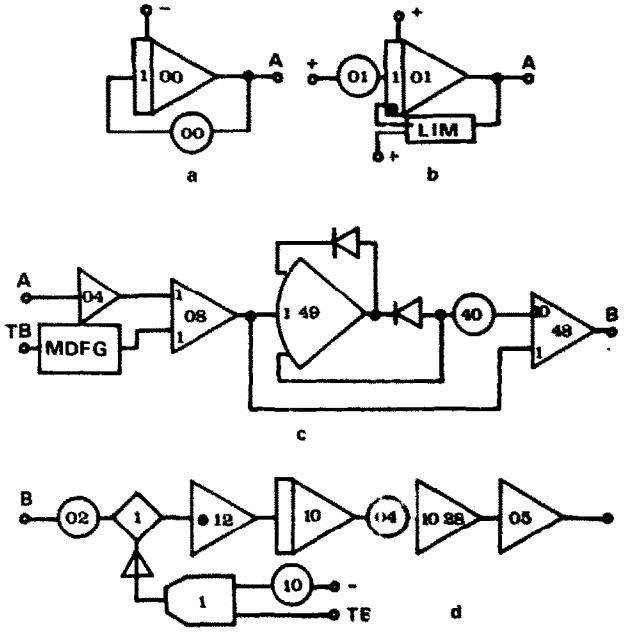

Fig. 1. Analog diagram for the treated procedure. With the aid of integrators in Fig. 1a and b the first- and zero-order kinetics are simulated, respectively. On the summer 08 in Fig. 1c the difference is obtained between the model response and the measured data, which are brought in through diode function generator (MDFG). With amplifiers 49 and 48, using two diodes, the absolute value of the difference is formed. Logically controlled switch 1 in Fig. 1d enables the integration of the optional part of the absolute difference curve on integrator 10. In our case the switch 1 is controlled by comparator 1 and potentiometer 10 to integrate only until the last measured point. Potentiometer 04 is used to scale the criterion function to the appropriate value to enable better comparison between criterion functions of the first- and zero-order kinetics.

Adaptive models can be successfully treated by an analog-hybrid computer. It is namely the most suitable for solving the system of differential equations, and at the same time it allows for variations of model structure and its parameters to be studied during the curve-fitting procedure. The treatment of stability data on an analog-hybrid computer starts with the introduction of experimental points through the diode function generator using linear interpolation. Model responses as the results of simulation of first- and zero-order degradation reactions are then obtained using integrators and potentiometers in appropriate combinations. Several criterion functions can be generated representing different ways of comparison between model responses and the experimental curve. In the present study, the integral of absolute value of the mentioned difference was chosen. The equation of the criterion function is presented in Eqn. 7, while the analog diagram for its generation is shown in Fig. 1.

$$F = c\int |D|dt \tag{7}$$

where F represents the criterion function, the expression |D| under integral represents the absolute difference between the curves of measured and simulated percentage of drug content, and c is a scaling constant. Discrete measured data are namely transformed into a continuous curve with the aid of linear interpolation.

Materials and Methods

Chemicals and solutions

Lorazepam (7-chloro-5-(2'-chlorophenyl)-1-,3-dihydro-3-hydroxy-2H-1,4-benzodiazepin-2-one) was obtained from KRKA, Pharmaceutical Industries, Novo Mesto, Yugoslavia. Quinazolinecarboxaldehyde derivative (6-chloro-4-(2'-chlorophenyl)-2-quinazolinecarboxaldehyde) was isolated and identified by the authors (Kmetec, 1983).

Potassium dihydrogenphosphate, p.a., sodium hydrogen phosphate, p.a., potassium chloride, p.a., methanol, p.a. were also used. Phosphate buffer pH = 7 was adjusted to constant ionic strength $\mu = 0.2$ by potassium chloride.

Conditions of storage

Lorazepam was dissolved in buffer solution to give a concentration 1×10^{-5} M. Two aliquots of this solution were exposed in Köttermann climatic test cabinet type 2306 at two different elevated temperatures namely 40 °C and 50 °C. Samples from the solution stored at 40 °C were withdrawn after 0, 5, 24, 68 and 164 h, while those stored at 50 °C were withdrawn after 0, 2, 7, 24, 68 and 164 h.

Analytical procedure

Samples were first diluted 10-fold with the mobile phase and then injected into high-performance liquid chromatographic system using:
apparatus—liquid chromatograph Varian LC 8500, spectrophotometer LC-UV Pye-Unicam, integrator Varian CDS 101, recorder Philips LC, injector Rheodyne 7125;

column—LiChrosorb 150 mm × 4.6 mm i.d., 5 μm RP 18;
mobile phase—methanol–0.01 M KH_2PO_4 (65 : 35);
conditions—room temperature, flow rate 60 ml/h, sensitivity 0.04 AUFS, wavelength 230 nm, loop volume 10 μl;
the *retention times* for lorazepam and quinazolinecarboxaldehyde derivative were 3.4 min. and 5.5 min., respectively.

Equipment for data evaluation

Microcomputer Apple II with recorder Epson FX-80 were used for linear regression and MSE analysis of experimental data. Analog-hybrid computer EAI 580 was used for simulation of lorazepam degradation reactions.

Results and Discussion

Time dependence of lorazepam and its degradation product quinazolinecarboxaldehyde derivative contents at declared experimental conditions are summarized in Table 1, where the contents are defined as percentages of initial lorazepam molar concentrations.

The results of the lorazepam experimental data evaluation by least-squares linear regression methods (Eqns. 1, 2, 3 and 6) together with appropriate Pearson correlation coefficients (Eqn. 4) and MSE values (Eqn. 5) are presented in Table 2.

From a theoretical point of view it is known that higher values of Pearson coefficient and lower values of MSE represent better curve-fitting. It is evident from Table 2 that the values of these criteria favour first-order lorazepam degradation kinetics at 50 °C. On the other hand, the results obtained at 40 °C show that an additional criterion(s) should be useful for uniform decision about the order of degradation reaction. The least-squares linear regression method namely favours zero-order, while the method of linear regression forced through 100% gives preference to the first-order degradation kinetics.

The applicability of analog-hybrid computer is illustrated by Figs. 2 and 3, where curves simulated for the first- and zero-order degradation kinetics are shown together with lorazepam experimental points and criterion function curves.

TABLE 1

THE PERCENTAGE OF LORAZEPAM (L) AND QUINAZOLINECARBOXALDEHYDE DERIVATIVE (Q) CONTENT AT DIFFERENT TIMES FOR 2 ELEVATED TEMPERATURES

T (°C)		Time (h)						
		0	2	5	7	24	68	164
40	L	100.0	-	96.5	-	92.1	83.7	62.1
	Q	0.0	-	2.7	-	4.9	10.0	23.4
50	L	100.0	96.3	-	92.9	84.5	62.0	26.1
	Q	0.0	1.8	-	4.0	23.0	29.8	39.8

TABLE 2

THE EQUATIONS OF REGRESSION LINES FOR ZERO- AND FIRST-ORDER LORAZEPAM DEGRADATION KINETICS WITH THE CORRESPONDING VALUES OF PEARSON COEFFICIENTS AND MEAN SQUARE ERROR (MSE)

T (°C)	Order of reaction	Line equation [a]	r	MSE [a]	Line equation [b]	MSE [b]
40	0	$y = 98.47 - 0.222t$	−0.9977	1.42	$y = 100.0 - 0.235t$	3.67
	1	$y = 4.60 - 0.0029t$	−0.9973	2.03	$y = 4.61 - 0.0029t$	2.68
50	0	$y = 96.47 - 0.442t$	−0.9951	9.75	$y = 100.0 - 0.471t$	21.63
	1	$y = 4.61 - 0.008t$	−0.9975	7.46	$y = 4.61 - 0.008t$	6.93

[a] Determined by the method of least-squares regression.
[b] Determined by the method of least-squares regression forced through 100%.

Comparison of criterion functions obtained by analog-hybrid simulation may represent the required parameter, which enables more objective decision about the order of degradation kinetics. The advantage of criterion function lies in its flexibility, which means that its time course can increase regularly or irregularly depending on the quality of experimental data. It is therefore not necessary that the final value of the function is absolutely minimized, because only the relative relationship between criterion functions for different-order reactions is important. However, considering some preliminary conditions concerning experimental data, the final value of the criterion function should be as low as possible. The result of the mentioned procedure gives an optimal value of the identified degradation rate constant.

As seen in Table 1, the data for quinazolinecarboxaldehyde derivative formation are given as well. The reason for this presentation is to show the additional

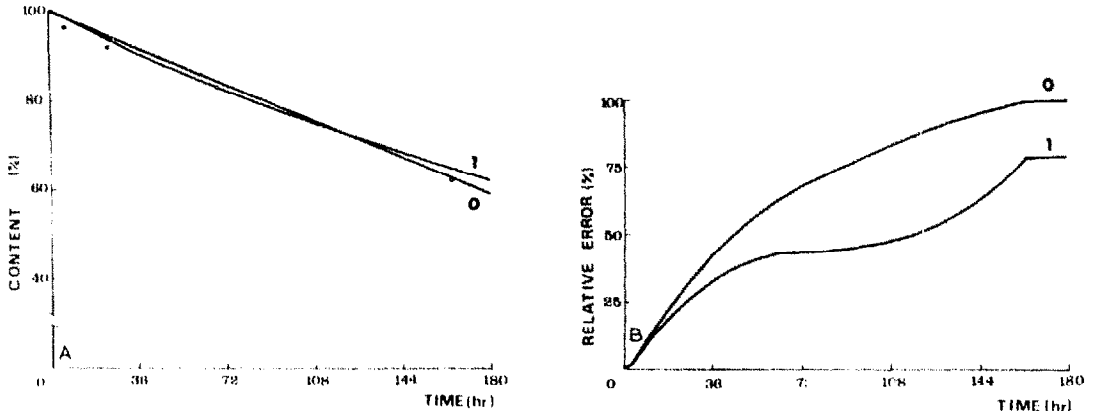

Fig. 2. A: simulated lorazepam content–time responses for zero-order (0) and first-order (1) degradation reaction (lines) and experimental data (circles) for 40 °C. Identified degradation rate constants are: $k_0 = 0.24\%\ h^{-1}$, $k_1 = 0.003\ h^{-1}$. B: criterion function time responses for zero-order (0) and first-order (1) simulated degradation reaction.

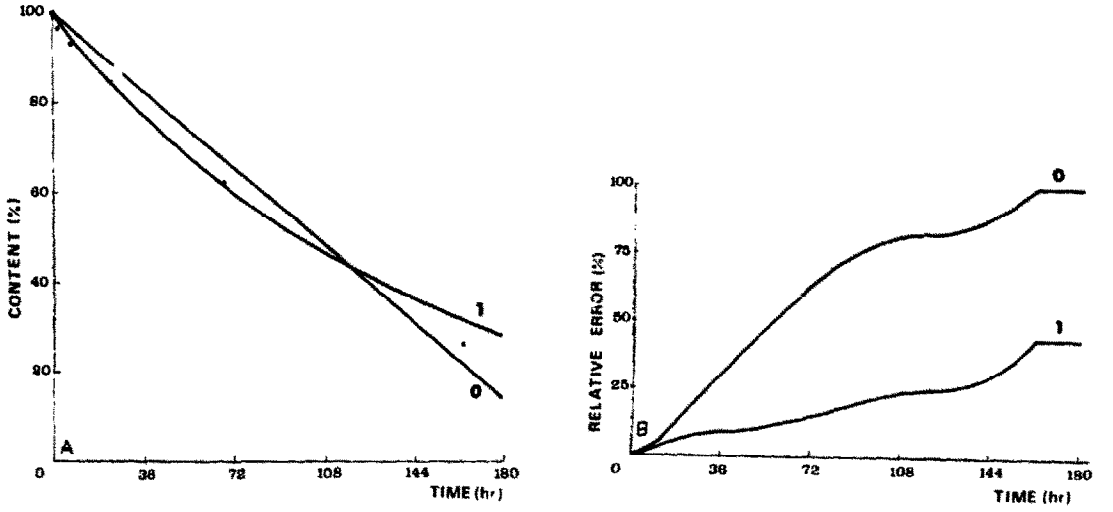

Fig. 3. A: simulated lorazepam content–time responses for zero-order (0) and first-order (1) degradation reaction (lines) and experimental data (circles) for 50 °C. Identified degradation rate constants are: $k_0 = 0.50\%\ h^{-1}$, $k_1 = 0.007\ h^{-1}$. B: criterion function time responses for zero-order (0) and first-order (1) simulated degradation reaction.

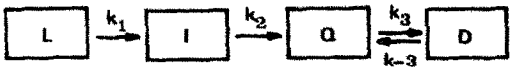

Fig. 4. Kinetic model for lorazepam degradation at the declared experimental conditions. The identified parameters are:
40 °C—$k_1 = 0.003\ h^{-1}$, $k_2 = 0.28\ h^{-1}$, $k_3 = 0.015\ h^{-1}$, $k_{-3} = 0.028\ h^{-1}$;
50 °C—$k_1 = 0.007\ h^{-1}$, $k_2 = 0.56\ h^{-1}$, $k_3 = 0.011\ h^{-1}$, $k_{-3} = 0.010\ h^{-1}$.
L = lorazepam; Q = quinazolinecarboxaldehyde derivative; I = intermediate; D = product of further reaction of quinazolinecarboxaldehyde derivative. The results of simulation are presented in Figs. 5 and 6.

Fig. 5.

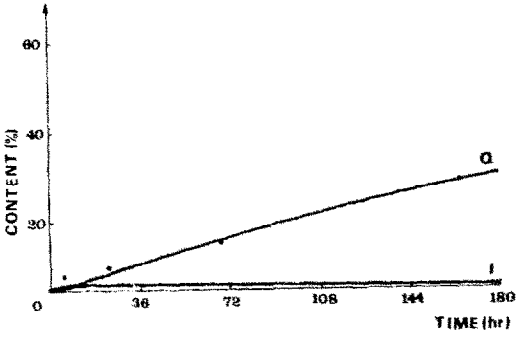

Fig. 6.

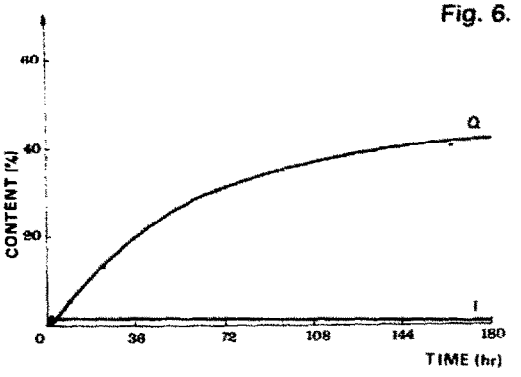

Fig. 5. Simulated quinazolinecarboxaldehyde derivative (Q) and intermediate (I) content–time responses (lines) for lorazepam degradation at 40 °C with experimental data for quinazolinecarboxaldehyde derivative (circles).

Fig. 6. Simulated quinazolinecarboxaldehyde derivative (Q) and intermediate (I) content–time responses (lines) for lorazepam degradation at 50 °C with experimental data for quinazolinecarboxaldehyde derivative (circles).

capabilities of analog-hybrid computer in the evaluation of drug stability. Using these experimental data and derived degradation rate constants, the kinetic model for lorazepam degradation pathways, shown in Fig. 4, was constructed using analog-hybrid simulation.

Treatment of the degradation products data by classical statistical means is often very difficult if not impossible. This is especially the case when extreme experimental conditions make the mechanism of drug degradation more complex. The analog-hybrid computer represents an effective tool for testing different kinetic models in a short period of time. Thus the most probable kinetic model from simulation as well as chemical point of view can be selected and the others rejected. The results of simulation, seen in Figs. 5 and 6, prove the suitability of the model in Fig. 4, assuming lorazepam degradation to quinazolinecarboxaldehyde derivative, which reacts further. Compartment D can namely represent the part of degradation mechanism by which quinazolinecarboxaldehyde derivative disproportionates to the corresponding carboxylic acid and alcohol (Rutgers and Shearer, 1981). At last the applicability of a developed model in a particular temperature range demonstrates the unchangability of the degradation mechanism and so the use of Arrhenius plotting in accelerated stability testing is justified.

References

Carstensen, J.T. and Su, K.S.E., Statistical aspects of Arrhenius plotting. Bull. Parenteral Drug Ass., 25/6 (1971) 287–302.

Carstensen, J.T., Theory of Pharmaceutical Systems, Academic Press, New York, 1972, pp. 17–23.

Karba, R., Bremšak, F., Matko, D., Mrhar, A. and Kozjek, F., Pharmacokinetic models—development and identification. In Preprints of the VIIth Triennial World Congress of the IFAC, Helsinki, 1978, pp. 557–564.

Karba, R., Bremšak, F., Mrhar, A., Kozjek, F., Matko, D. and Kmetec, V., Analog-hybrid simulation in nonlinear pharmacokinetical modelling. In Proceedings of IInd World Conference on Mathematics at the Service of Man, Las Palmas, 1982, pp. 366–369.

Kmetec, V., Kozjek, F. and Karba, R., Analog-hybrid simulation in drug stability. In Proceedings of IIIrd Mediterranean Conference on Biomedical Engineering, Portorož, 1983, p. 13.5.

Kmetec, V., Master's Degree Thesis, University of E. Kardelj, Ljubljana, 1983, pp. 53–55.

Rutgers, J.G. and Shearer, C.M., Lorazepam Analytical Profiles of Drug Substances, K. Florey (Ed.) Vol. 9, 1981, pp. 411–413.

Wagner, J.G., Fundamentals of Clinical Pharmacokinetics, Drug Intelligence, Hamilton, 1975, pp. 285–290.

Yang, W., Statistical treatment of stability data. Drug Develop. Ind. Pharm., 7 (1981) 63–77.

Young, J.F., White, C.G. and Pearce, B., A hybrid computer system for pharmacokinetic modelling. II. Applications. In Proceedings of the 1981 Summer Computer Simulation Conference, 1981, pp. 122–129.