original sample.

Although the method outlined is an independent one, which does not require prior knowledge of possible interfering substances, care must be taken to ensure its generality. The inclusion of just two wavelengths in the detection method, as in Eq. 2, ignores absorbances from contaminants at all other wavelengths. A material which has the same ratio of absorbances at these two wavelengths as the drug-substance (or zero absorbance at each) would not be detected, regardless of the nature of its absorbances at other wavelengths. To enable the detection of the widest range of interfering substances, absorption values across the entire spectral region of interest should be compared. This can be accomplished by either sequential application of the simple relationship of Eq. 2, varying the wavelengths each time, or by using an extended expression which includes the absorbances at several wavelengths.

The latter approach is illustrated in Fig. 7 for the carbamazepine system. Figure 7A shows the unresolved chromatographic peak due to carbamazepine and acridone (1%). To detect the acridone, the multiwavelength expression of Eq. 12 was plotted *versus* retention time:

$$\overline{A}_{243\text{ nm}-245\text{ nm}} + \overline{A}_{266\text{ nm}-268\text{ nm}} - \overline{A}_{289\text{ nm}-290\text{ nm}} - \overline{A}_{301\text{ nm}-302\text{ nm}} = 0 \quad \text{(Eq. 12)}$$

For simplicity, wavelength ranges were chosen whose absorbance values will cancel without the use of proportionality constants. As shown in Fig. 7B, the results are comparable in terms of sensitivity to those given in Fig. 1, and the cancellation of the response due to pure carbamazepine is equally complete (Fig. 7C). The major benefit of this approach is in the generality obtained in one operation. A standard method, which includes sufficient wavelengths to be of wide scope, would be the use of absorbances at 20 nm intervals across a broad spectral region (~200 nm).

One concern is the degree of homogeneity indicated in these methods by a flat baseline. Two limitations imposed by the apparatus described under *Experimental*, are the experimentally determined response threshold of ±0.001*A* and the photometric linearity constraint. This latter restriction requires maximum absorbance values of ~1.0 *A* for wavelengths used in the null relationship, to preserve optimum photometric linearity. Thus, the maximum amount of drug substance analyzed, which governs the relative level of impurities found, is determined by this photometric limitation and by the chromatographic constraint against overloading the column. Given these instrumental restrictions, there are three principal factors which affect the response level of a given impurity: the intensity of its absorption (molar absorptivity), the degree of similarity between its absorption spectrum and that of the drug substance, and the detection method (null relationship) employed. In the case of unknown materials, absorption characteristics are unknown quantities, and the detection method cannot be tailored to attain the maximum response. Because of this, the use of multiple expressions containing data obtained at several wavelengths is recommended. An *a priori* detection limit cannot be stated for all possible impurities. However, these methods can ensure >99% homogeneity in most cases.

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Kinetics and Stability of a Multicomponent Organophosphate Antidote Formulation in Glass and Plastic

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Abstract □ An aqueous solution of trimedoxime bromide, atropine, and benactyzine hydrochloride was formulated to have maximum stability as an antidote in organophosphorus poisoning. The stability of the mixture in glass and plastic cartridges was determined. Glass cartridges were more desirable than plastic; there was less vapor loss, color formation, and anomolous reaction. Trimedoxime was stable, losing 1.4% of its potency after 1 year at 25° and atropine was more stable than trimedoxime. Considerable degradation of benactyzine occurred; 20% of its potency was lost after 1 year at 25°. Equations for predicting the shelf life of each ingredient at selected temperatures are presented.

Keyphrases □ Benactyzine—in formulation, kinetics and stability in glass and plastic □ Atropine—in formulation, kinetics and stability in glass and plastic □ Trimedoxime—in formulation, kinetics and stability in glass and plastic

The administration of atropine with and without oxime is a common therapy for poisoning by organophosphorus anticholinesterase pesticides such as parathion[1] and malathion[1], and for other organophosphorus compounds such as isopropylmethylphosphonofluoridate (sarin) and pinacolyl methylphosphonofluoridate (soman). Atropine is used to overcome cholinergic stimulation from the anticholinesterases, and oxime reactivates and restores the activity of the enzyme. A recent report claims that the effectiveness of a therapy could be enhanced significantly by simultaneous administration of the cholinolytic drugs,

[1] Parathion, *O,O*-diethyl-*O*-(4-nitrophenyl)phosphorothioate; malathion, *O,O*-dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate; trimedoxime bromide, pyridinium-1,1′-(1,3-propanediyl)bis(4-(hydroxyimino)methyl)-dibromide; and benactyzine, α-hydroxy-α-penylbenzeneacetic acid 2-(diethylamino)ethyl ester.

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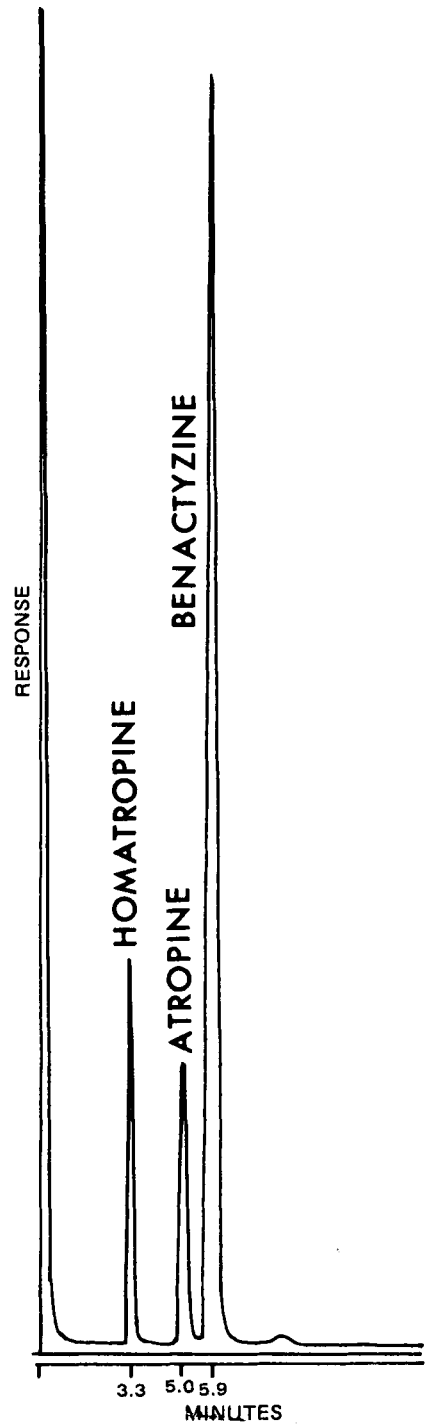

Figure 1—*Chromatogram of atropine and benactyzine with the internal standard, homatropine.*

atropine, and benactyzine[1] (1). This investigation was initiated in answer to the need of the United States Defense Forces for a stable and ready-for-use antidote that would be effective against a variety of organophosphorus compounds. Studies were conducted with a three-drug combination containing trimedoxime bromide[1], atropine sulfate, and benactyzine hydrochloride. The stability of aqueous solutions of the multicomponent mixture, in prefilled glass or plastic cartridges for a semiautomatic injector device, was studied.

The kinetics of hydrolysis of atropine and trimedoxime bromide in dilute aqueous solution in the pH range 0.5–

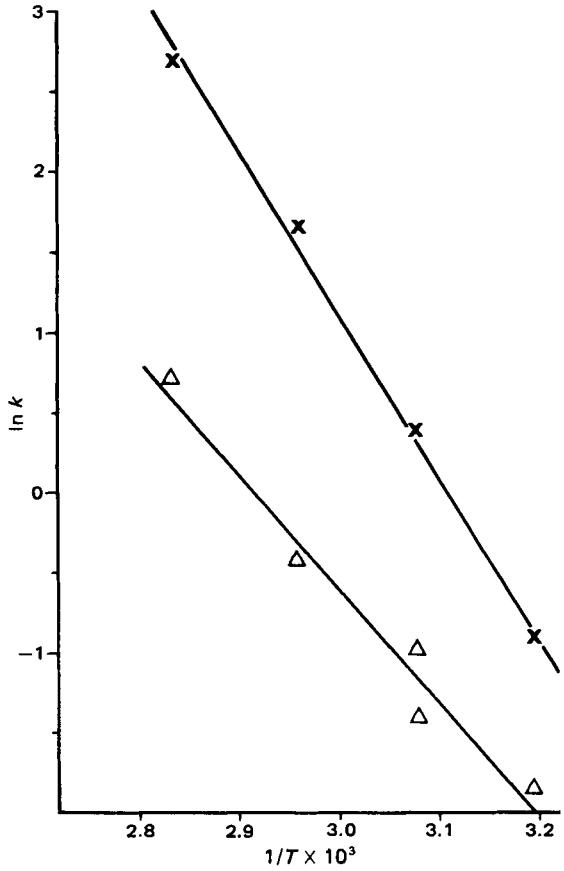

Figure 2—*Effect of temperature on net weight loss. Key: △, glass cartridges; ×, plastic cartridges.*

13.0 and at various temperatures were reported previously (2–4). Another report (5) studied the stability of benactyzine in the pH range 2.1–6.3 at 37°. A mixture of the three drugs (trimedoxime bromide, atropine sulfate, and benactyzine hydrochloride) was prepared at pH 2.8 to maximize the overall stability of the active ingredients of the multicomponent formulation.

EXPERIMENTAL

Materials—Cartridges were either glass (USP type 1) or polypropylene resins. Cartridge seals were butyl rubber.

The ingredients in the cartridge were: trimedoxime bromide, 20 mg/ml; atropine sulfate USP, 0.5 mg/ml; benactyzine hydrochloride, 2.05 mg/ml; methylparaben USP, 0.5 mg/ml; and propylparaben, 0.05 mg/ml[2]. All ingredients are dissolved in USP Water for Injection. Dilute hydrochloric acid USP, ~0.5 ml/liter, was used to adjust the pH to 2.8. Each cartridge was filled to 2.0 ml. The open ends of the cylindrical cartridges were stoppered with tight-fitting gray butyl rubber plugs.

Storage—The glass and plastic cartridge assemblies were wrapped individually in aluminum foil. They were then placed in a horizontal position in constant temperature ovens at either 80° for 3 weeks, 65° for 7 weeks, 52° for 3 months; in a water-jacketed incubator at 40° for 4 months; or in a refrigerator at 5° for 4 months. Some assemblies stored at 65° were placed vertically to determine whether the storage position had any influence. The physical appearance of the assembly cartridges, the net weight of the contents, pH, and the concentrations of benactyzine, trimedoxime, and atropine were studied. Samples were removed from storage at intervals for assessment and analysis. Differences found between glass and plastic cartridges were evaluated using a t test to determine significance at 95% confidence limits (6). The data for both types

[2] Reference standards of trimedoxime bromide and benactyzine hydrochloride are available upon request to Contracting Officer, Defense Personnel Support Center, Directorate of Medical Material, 2800 South 20th Street, Philadelphia, PA 19401.

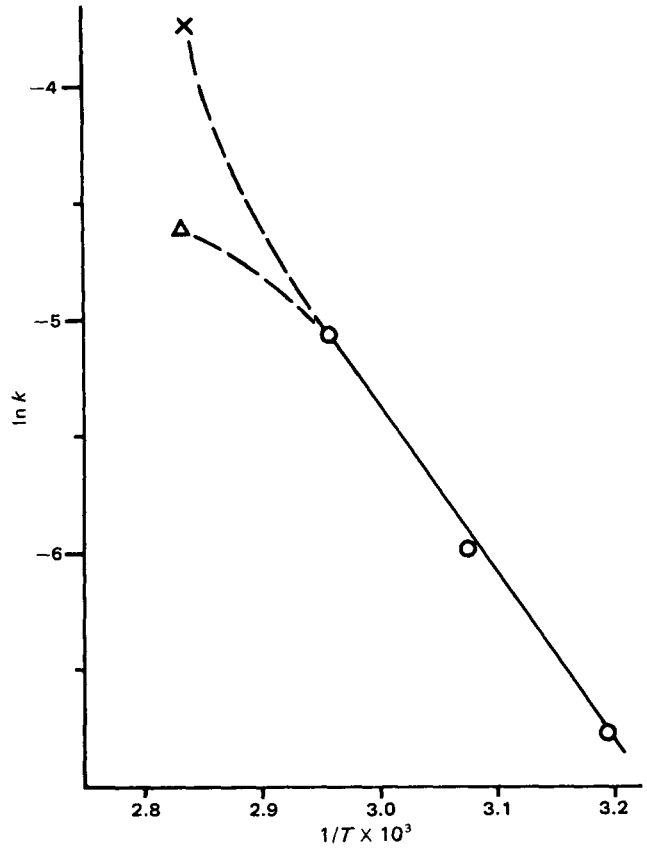

Figure 3—*Influence of temperature on pH decrease. Key: △, glass cartridges; ×, plastic cartridges. Values at 80° deviate significantly from the Arrhenius plot.*

of cartridges were combined where no statistically significant difference was shown.

Assay—*Benactyzine Hydrochloride and Atropine Sulfate*—One milliliter of each antidote mixture was pipetted into 10-ml glass tubes with polytef-lined screw caps. Internal standard[3] (1.0 ml), chloroform (1.0 ml), and 7% sodium carbonate (1.0 ml) were added successively. The tubes were capped immediately, shaken vigorously for 2 min, and the layers were allowed to separate. Approximately 2–3 μl of the chloroform layer was injected into the chromatograph.

The gas chromatographic analysis was done on a 1.2 m × 4-mm i.d. glass column with a 100/120-mesh Gas Chrom Q with 3% OV-17 column support and coating. The column temperature was 200° isothermal, the injection port temperature 190°, and the detector temperature 220°. The carrier gas was helium at a flow rate of 80 ml/min. The detector had an 80 ml/min hydrogen flow, and a 450 ml/min air flow. A typical chromatogram is shown in Fig. 1.

Trimedoxime Bromide (*4*)—Fifty microliters of the cartridge solution was added to 14.0 ml of deionized water; 100 μl of this dilution was added to 10.0 ml of 0.05 *N* NaOH and the resulting mixture was scanned between 400 and 300 nm. The absorbance at the peak near 346 nm was compared to that of a standard solution and the trimedoxime concentration was determined. In a series of samples, a blank and a standard solution were run at the beginning and end of the series. In larger groups, a standard solution was also interspersed between every 8–10 samples. The standard solution was 20 mg of trimedoxime/ml, 0.05% methylparaben, and 0.005% propylparaben in 10^{-3} *M* HCl. This solution was filtered through a 0.22 μm filter and stored at 5°.

RESULTS

The cartridges stored at 5° were assumed to yield baseline values. Over the period of the study there were no changes in the concentrations of trimedoxime, atropine, and benactyzine at 5°. The solutions from these cartridges were clear and faintly yellow, a color characteristic of trimedoxime solutions. No statistically significant differences between the glass and plastic cartridges were demonstrated at this temperature for any of

[3] Homotropine hydrobromide USP, 0.5 ng/ml in 10^{-3} *N* hydrochloric acid.

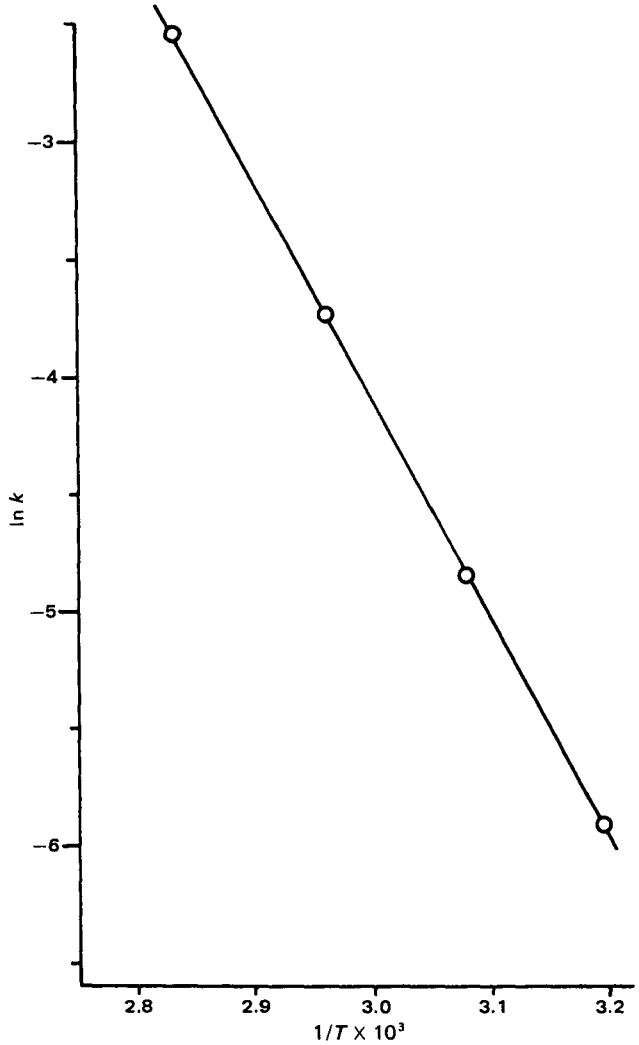

Figure 4—*Influence of temperature on the degradation rates of benactyzine.*

the factors studied except net weight. The average weight of the ejected contents for the plastic cartridge was 14 mg less than that for the glass cartridge at 5°.

The solutions stored at elevated temperatures in both the glass and the plastic cartridges remained clear throughout the study. Solutions in glass cartridges developed a trace of yellow color at 80°. Solutions in the plastic cartridge developed color much faster. At the end of each storage period the solutions were bright yellow at 80°, moderately yellow at 65°, light yellow at 52°, and at 40° contained a trace of yellow. One sample at 40° was very dark yellow, and had a pH of 2.5, (~0.2 units lower than expected).

No physical change was observed in glass cartridges throughout the study. In several plastic cartridges stored at 80°, the pressure generated was sufficient to move the rubber plunger toward the open end of the cartridge. The plastic cartridges became stained with a narrow brown ring at the interface of the plunger and the solution. In the cartridges where the plunger had moved, the stain was diffused over the path of the plunger movement. The stain was apparent in less than 1 week at 80 and 65°, in ~1 month at 52°, and in ~4 months at 40°.

No significant differences were demonstrated when the cartridges were stored in a vertical position (either end up) at 65° when compared with those stored in a horizontal position.

Reaction velocities of the various stability-related factors are influenced by temperature. This is expressed by the Arrhenius equation in its integrated form:

$$\ln k = A - E/RT \qquad \text{(Eq. 1)}$$

where k is the specific rate constant; E is the energy of activation; R is the gas constant (1.987 cal/deg mole); T is the absolute temperature; and A is a frequency factor.

The data were used to determine the best value of k at each tempera-

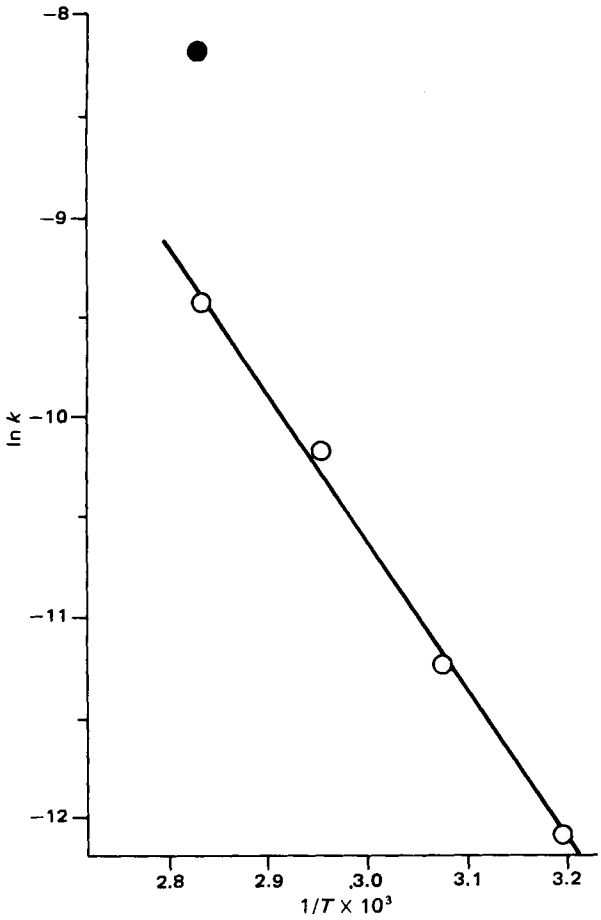

Figure 5—*Influence of temperature on the degradation rates of trimedoxime. The value for plastic cartridges at 80° (●) deviates significantly from the Arrhenius plot.*

ture. The best line of ln k *versus* $1/T$ was fitted by the least-squares method and the intercept (A) and the slope ($-E/R$) were determined.

Weight Loss—Loss of water from the solution in the cartridges, presumably occurring through the rubber closures and the walls of the plastic cartridge, would be reflected in a change in volume. Since the loss was small, measuring the change in weight was considered a more sensitive method of determining the loss than measuring the volume differences. The rate of weight loss in the glass and plastic cartridges was determined at each temperature, using the equation:

$$w = w_0 - kt \qquad \text{(Eq. 2)}$$

where w_0 is the weight in milligrams at the zero intercept, k is the rate of weight loss in milligrams per day[4]; and t is the time in days.

The effect of temperature on weight loss was significantly different between the two types of cartridges. The activation energies for the weight loss in the glass and plastic containers were 14.1 ± 1.6 (SE) and 20.1 ± 0.9 (SE) kcal, respectively. The Arrhenius plot for the glass and plastic cartridges is shown in Fig. 2.

Change in pH—The pH of the solutions in the cartridges decreased with storage time and can be expressed by the equation:

$$\text{pH} = \text{pH}_0 - kt \qquad \text{(Eq. 3)}$$

where pH_0 is the pH at time zero; k is in pH units per day; and t is time in days.

The rate of decrease in pH was significantly different between the glass and plastic cartridges at 80°. At this temperature the rate was lower for the glass cartridge than would be predicted from the other three temperature points, and higher for the plastic cartridge. No significant pH differences between glass and plastic cartridges were found at the lower temperatures. The best values for k are plotted in Fig. 3 and are expressed by the equation:

[4] The unit for k is generally expressed in concentration rather than weight. The authors feel that by expressing water loss in weight, rather than concentration, the data would be more meaningful.

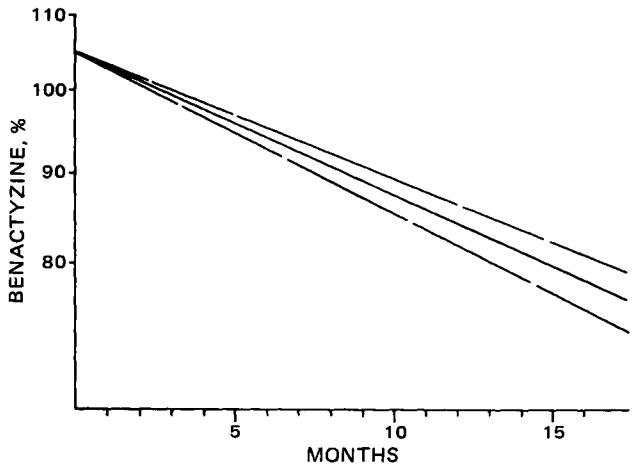

Figure 6—*Predicted disappearance of benactyzine hydrochloride at 25°. The solid line is the predicted thermal degradation rate of benactyzine in the injector at 105% fill. The dashed lines encompass the 95% confidence limits of the predicted rate.*

$$\ln k = 16.1455 - 14{,}300/RT \qquad \text{(Eq. 4)}$$

where the units for k are pH units/day. The standard error for the energy of activation is 690 calories.

Active Ingredients—*Benactyzine Hydrochloride*—The degradation of benactyzine in the mixture can be described adequately by the first-order rate equation:

$$\ln C = \ln C_0 - kt \qquad \text{(Eq. 5)}$$

where C is in milligrams per milliliter, C_0 is concentration at zero time, and t is time in days. There were no statistically significant differences between the glass and plastic cartridges for either C_0 or k.

The effect of temperature on the degradation rate is illustrated in Fig. 4, where the equation is expressed by:

$$\ln k = 23.7461 - 18{,}460/RT \qquad \text{(Eq. 6)}$$

where the unit of k is day^{-1} and the standard error for the activation energy is 150 calories.

Atropine Sulfate—No statistically significant disappearance of atropine sulfate in either glass or plastic cartridges could be demonstrated at any temperature during the term of this study.

Trimedoxime Bromide—The decrease in trimedoxime concentration is expressed by the zero-order equation:

$$C = C_0 - kt \qquad \text{(Eq. 7)}$$

where C is in moles per liter, C_0 is the concentration at zero time, k is in milligrams per day, and t is time in days.

A significant difference between the k values for glass and plastic

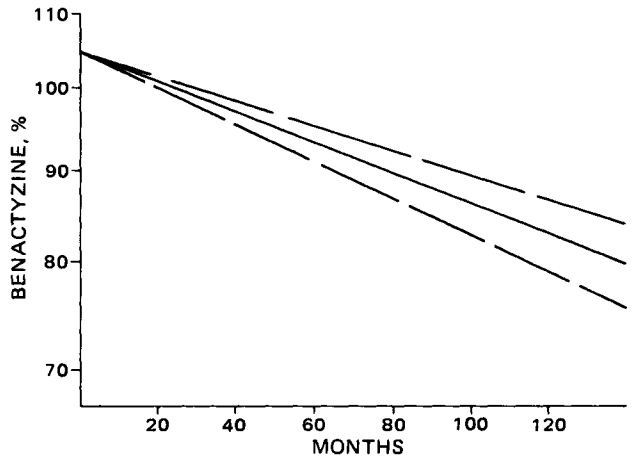

Figure 7—*Predicted disappearance of benactyzine hydrochloride at 5°. The solid line is the predicted thermal degradation rate of benactyzine in the injector at 105% fill. The dashed lines encompass the 95% confidence limits of the predicted rate.*

Table I—Predicted Shelf Life of Benactyzine in Multicomponent Formulation

Temperature	90% Shelf Life	75% Shelf Life
5°	4.5 years	12.3 years
15°	1.4 years	3.9 years
25°	5.9 months	1.3 years
40°	1.3 months	3.6 months
60°	0.2 months	0.6 months

cartridges was found only at 80°. No significant differences were found with regard to k or C_0 at other temperatures. The data for plastic cartridges at 80° were excluded in calculating the regression equation in Fig. 5. The line is expressed by

$$\ln k = 18.0100 - 14{,}900/RT \qquad \text{(Eq. 8)}$$

where k is in moles per liter-day. The standard error for the activation energy is 880 calories.

DISCUSSION

Glass cartridges were found to be more suitable than plastic as containers for trimedoxime, atropine, and benactyzine formulations. Benactyzine is the least stable of the active ingredients in the antidote formulation. Results of this study show that benactyzine degrades ~20% after 1 year at 25° in either plastic or glass. The usefulness of the injector could be prolonged by storage at 5°, where the predicted decrease in benactyzine is 10% after 5 years. Figures 6 and 7 illustrate the enhanced shelf life of benactyzine when filled at 105% of label and stored at 25 or 5°. Predicted shelf life at various temperatures is presented in Table I.

The trimedoxime in the injector is very stable; about 1.4% degradation would occur after 1 year at 25°. Solutions of trimedoxime can exist in equilibrium with its hydrolysis products (7, 8). The degradation rate of trimedoxime in plastic cartridges at 80° was faster then expected. The unusual drop in pH at 80° (Fig. 3), greater than expected based on temperature studies, could shift the equilibrium of trimedoxime and consequently account for the increased degradation rate in plastic.

Atropine sulfate in the medication is quite stable. According to kinetic studies of atropine in solution at pH 2.8, its half-life would be about 200 years at 25° and about 2 years at 80° (2, 3).

The pH values in glass were homogeneous at all temperatures. There was some heterogeneity of pH in the plastic cartridge; *i.e.*, 2 of 23 samples stored at 5° had a pH of about 2.95; one sample at 80° had a pH of 3.01, 0.5 units more than expected. A sample at 40° had a pH of 2.51, 0.2 units lower than expected. The decrease in pH with storage time was consistent with temperatures of 65° and below. At 80°, the decrease in pH was slower than expected, suggesting abstraction of hydrogen ion by the rubber in the cartridge. In the plastic cartridge, the pH decreased at a considerably faster rate than expected; this observation suggests a reaction caused by some material in plastic at this temperature. At 25° the drop in pH, probably due to the formation of benzilic acid, is predicted to be less than 0.15 units after 1 year of storage.

Vapor transmission from the cartridges at ambient temperature is low; it is estimated that water loss would be about 1%/year for the glass cartridge and about 2%/year for the plastic cartridge at 25°.

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Factors Influencing the Apparent Protein Binding of Quinidine

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Abstract □ Various factors influencing the apparent protein binding of quinidine were examined. Different binding values in rabbit plasma were obtained by equilibrium dialysis techniques employing three commonly used buffers. Binding values comparable to those found by ultrafiltration were achieved after dialysis against isotonic phosphate buffer for ~4 hr. Dialysis beyond 8 hr gave an increased free fraction with time. The reported effect of *in vitro* added heparin on plasma protein binding could be prevented by reducing the final concentration in blood from 20 to 5 U/ml, a concentration still sufficient to prevent clotting of the blood sample. Daily freezing and thawing of plasma samples over 1 week did not alter the binding of quinidine. The samples were stable for at least 2 months at −20°.

Keyphrases □ Binding, protein—quinidine, factors influencing binding, rabbit plasma □ Quinidine—factors influencing protein binding, rabbit plasma □ Plasma protein binding—quinidine, factors influencing binding, rabbits

A wide range of protein binding values for quinidine have been reported. Mean unbound fractions of 0.10–0.29 were observed in normal volunteers (1–5), while mean values of 0.19–0.42 were found for patients with cirrhosis (2, 3). Although interindividual differences in binding properties may contribute significantly to the variability in these results, differences in the blood collection method and in the binding determination could be important contributing factors. Ultrafiltration and equilibrium dialysis have been used previously for quinidine binding determinations. In equilibrium dialysis, various investigators have used different temperatures, equilibrium

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