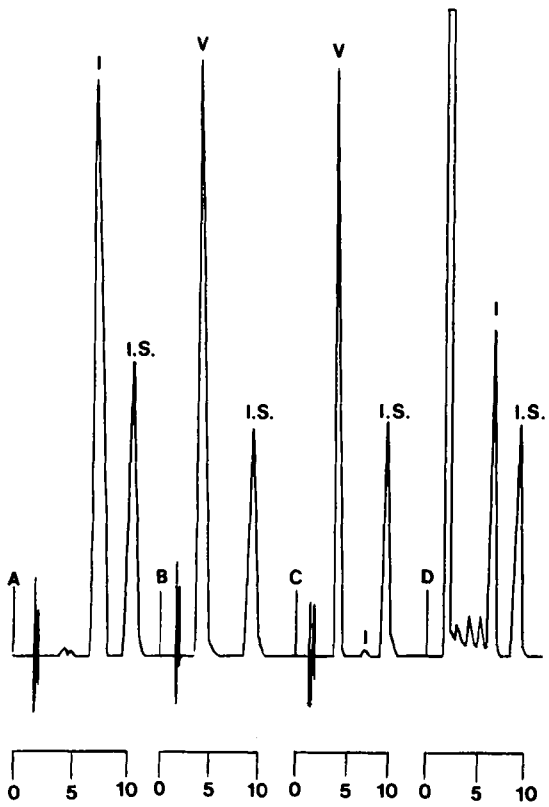

Figure 2—*Chromatogram of esmolol hydrochloride solutions boiled for 1 h. Key: (A) water, pH 5.5; (B) 1 M NaOH; (C) 1 M HCl; (D) 30% H_2O_2.*

HCl or 10 M NaOH. All flasks were brought to volume with water and analyzed.

RESULTS AND DISCUSSION

The direct measurement of the raw drug for esmolol hydrochloride content in the presence of the anticipated synthetic intermediates, the synthetic starting material, and the anticipated breakdown product is shown in Fig. 1. The detector wavelength (280 nm) was chosen to enhance visualization of all potential synthetic intermediates and not for maximum sensitivity for esmolol. The limit of quantitation for esmolol hydrochloride was ~10 μg/mL under the reported operating conditions.

Table I—Analysis of Four Experimental Lots of Esmolol Hydrochloride

Lot	Mean, %	RSD, %	Number of Determinations	Time
A	97.8	1.15	24	2 years
B	97.4	1.23	24	2 years
C	98.6	1.95	21	1 year
D	97.5	0.81	18	9 months

Applicability—The specificity of the HPLC system was tested with degraded esmolol samples. No changes in I concentration were seen in the boiled aqueous solution. After 1 h in boiling acid (1 M HCl) or base (1 M NaOH), I was almost completely converted to V, as might be expected under these conditions. Finally, boiling for 1 h in 33% hydrogen peroxide yielded several additional unidentified products (Fig. 2). In each chromatogram, it can be seen that the size of the I peak decreases with degradation. The practicality of the method was demonstrated by the analysis of four synthetic lots (Table I). The percent *RSD* values for the analysis over a 2-year period and with several analysts were consistently <2%.

Accuracy and Precision—In spite of consistently high correlation coefficients (>0.996), the peak height ratio method was not employed, as erroneous results were obtained due to tailing at higher concentrations. Curvature or tailing did not influence the peak area ratio calculations which were employed for all studies. Data generated by three separate analysts on each of 3 d yielded an accuracy >98.6%, percent *RSD* of <1.64%, and correlation coefficients >0.9996 for the calibration curves. The percent *RSD* values for a single sample were <2% (Table I).

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Effect of Ethanol, Glycerol, and Propylene Glycol on the Stability of Phenobarbital Sodium

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Abstract □ The effects of ethanol, glycerol, propylene glycol, phosphate buffer, and ionic strength on the stability of phenobarbital sodium have been studied. Ethanol had the maximum stabilization effect followed by propylene glycol and glycerol when compared with the stability in water. The estimated half-lives at 50°C (pH ~ 8) were 78, 95, 109, and 127 d in water and 20% aqueous solutions of glycerol, propylene glycol, and ethanol, respectively. The effects of phosphate buffer and ionic strength were negligible.

Keyphrases □ Phenobarbital sodium—stability, effects of ethanol, glycerol, and propylene glycol □ Stability—phenobarbital, effect of solvents

It is well known that the stability of phenobarbital in liquid dosage forms depends on the pH and the vehicle. A common method to minimize degradation (1) is to use a mixed solvent of water and an organic solvent such as ethanol, glycerol, or propylene glycol. The stabilization effect of ethanol is thought to be due to a decreased dielectric constant (2), which slows down the reaction between ions of like charges, *i.e.*, the ionized form of phenobarbital and the hydroxyl ions.

An earlier report (1) indicated that it was difficult to select a stability-indicating method for the quantitation of phenobarbital. Recently, a stability-indicating assay method (3) based on HPLC has been reported which is applicable to liquid

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Table I—Stability Studies of Phenobarbital Sodium Solutions (0.5 mg/mL)

Solution	Solvent[a]	Phosphate Buffer, M	Ionic Strength[b]	Apparent $pH_{initial}$ (±0.1)	Estimated Half-Life at 50°C, d	Apparent pH_{final}[c] (±0.1)
1	—[d]	0.1	0.26	7.7	78	7.8
2	20% Ethanol	0.1	0.26	8.1	127	8.0
3	40% Ethanol	0.1	0.26	8.4	170	8.2
4	20% Glycerol	0.1	0.26	7.7	95	7.6
5	40% Glycerol	0.1	0,.26	7.7	134	7.6
6	20% Propylene glycol	0.1	0.26	7.9	109	8.0
7	40% Propylene glycol	0.1	0.26	8.2	165	8.2
8	—[d]	0.05	0.39	7.7	79	7.8
9	—[d]	0.1	0.39	7.7	77	7.8
10	—[d]	0.15	0.39	7.7	77	7.8
11	—[d]	0.1	0.26	7.7	78	7.8
12	—[d]	0.1	0.39	7.7	79	7.8
13	—[d]	0.1	0.52	7.7	80	7.8

[a] Prepared in water. [b] Adjusted with KCl. [c] After 163 d. [d] Water alone.

dosage forms such as phenobarbital elixir. The purpose of these investigations was to study the effect of ethanol, glycerol, propylene glycol, phosphate buffer concentration, and ionic strength on the stability of phenobarbital sodium using the recently developed (3) HPLC method.

EXPERIMENTAL SECTION

Chemicals, Reagents, and Apparatus—All chemicals and reagents were either USP, NF, or ACS quality. Phenobarbital sodium[1] (1) was used as received.

The chromatograph[2] was equipped with a multiple-wavelength detector[3] and a recorder[4]. A semipolar column[5] (30 cm × 4 mm i.d.) was used. The mobile phase contained 35% methanol in 0.02 M aqueous ammonium acetate. The flow rate was 3.0 mL/min, and the temperature was ambient. The sensitivity was set at 0.1 (245 nm) and the chart speed was 30.5 cm/h.

Preparation of Phenobarbital Sodium Solutions—All solutions (Table I) contained 0.5 mg/mL of phenobarbital sodium and were prepared using a simple solution method. The standard solution of phenobarbital sodium in water (0.5 mg/mL) was prepared fresh daily.

After the initial assay and pH value determination[6] the solutions were stored at 50 ± 1°C in 60-mL amber bottles[7] in an electric oven. The data were recorded again at appropriate intervals. The solutions were assayed using HPLC (3) except that the flow rate was 3.0 mL/min. The other chromatographic conditions were the same, except that no internal standard could be added since the solutions were injected into the chromatograph without dilution. The results were calculated using a previously described equation (3) and are presented in Table I and Fig. 1.

RESULTS AND DISCUSSION

Effect of Vehicle—The results (Table I) indicate that ethanol had the maximum stabilizing effect on phenobarbital sodium followed by propylene glycol and glycerol. The apparent pH values of these solutions varied slightly, although they all contained 0.1 M phosphate buffer and the ionic strength was adjusted to 0.26 with KCl. The pH-rate profile curve of phenobarbital is less sensitive to pH changes from 7.5 to 9 (4), the range used in these experiments.

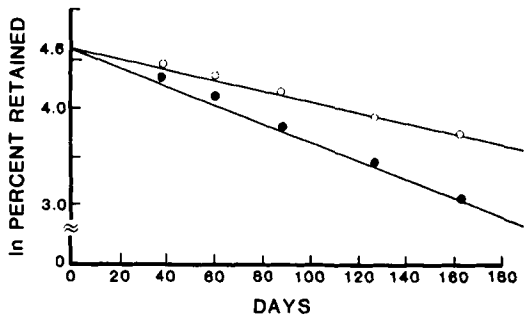

Figure 1—*First-order plots of solutions 1 and 5 (Table I). Key: (●) solution 1; (○) solution 5.*

Above pH 9, decomposition occurs readily (4). Considering this, it is obvious that the stabilizing effect of ethanol may be even better than the results indicate since the initial pH values of solutions containing ethanol (Table I) were higher than those containing propylene glycol or glycerol. Solutions containing glycerol had the lowest apparent pH values (Table I) and still had less stabilizing effect than propylene glycol and ethanol. Glycerol was better only when compared with water (Table I), which was expected. The estimated half-lives of solutions in water, 20% glycerol, 20% propylene glycol, and 20% ethanol were 78, 95, 109, and 127 d, respectively. Obviously, the stabilizing effect is inversely related to dielectric constant values of 80, 43, 32, and 25 for water, glycerol, propylene glycol, and ethanol, respectively, at 20°C. This is in agreement with earlier observations (2) that the stabilization effect is due to a decrease in the dielectric constant, which slows down the reaction between ions of like charges. Using the first-order equation (Fig. 1) and taking the activation energy value of 25 kcal/mol-deg (4), the t_{90} for solution 7 (Table I) at 25°C was determined to be ~1.8 years.

Effect of Phosphate Buffer and Ionic Strength—The phosphate buffer did not catalyze the reaction (solutions 8–10, Table I) and the effect of ionic strength was negligible (solutions 11–13). This was expected since the reaction between the un-ionized molecule of phenobarbital and the hydroxyl radical is ~250 times greater than that between the ionized form and OH^- (4).

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