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Technical Note

Phenobarbital Stability in Different Dosage Forms: Alternatives for Elixirs

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INTRODUCTION

The only oral solution dosage form of phenobarbital currently marketed is an elixir. An elixir is a hydroalcoholic dosage form used as a vehicle because ethanol enhances solubility and promotes the stability of phenobarbital by reducing the rate of hydrolysis of the ionized species (1,2). Advantages over solid dosage forms may include increased bioavailability and ease of administration.

The use of alcohol may, however, present problems in drug therapy because of the synergistic action of alcohol with other drugs. These therapeutic problems are of particular concern in elderly patients (3–5). Emulsions may possess the same advantage of other liquid dosage forms while eliminating the alcohol.

The present study evaluated the stability of phenobarbital as an elixir, emulsion, aqueous solution, and aqueous solution with propylene glycol.

EXPERIMENTAL

Preparation of Dosage Forms

Phenobarbital Emulsions. Cholesterol (100 mg), phenobarbital (400 mg), Span 85 (1 ml), and corn oil (30 ml) were added to a flask, stirred, and heated (70°C) until all the phenobarbital was dissolved. Sorenson buffer (65 ml, 2/15 *M*, pH 5) containing 40% (v/v) propylene glycol (final pH 5.4) was added to Tween 85 (2 ml). The aqueous and oil phases were heated to 70°C and mixed. The preparation was cooled to room temperature and adjusted to 100 ml with Sorensen buffer, shaken, heated to 70°C, and homogenized three times with a hand homogenizer. A blank emulsion containing propylene glycol was also prepared. Another phenobarbital emulsion was also prepared in the same manner without propylene glycol.

Phenobarbital Solutions. A concentration of sodium phenobarbital equivalent to the concentration of the free acid used in the emulsions was used. Sodium phenobarbitral (438 mg) was diluted to 100 ml with Sorenson buffer (2/15 *M*, pH 5) containing 40% (v/v) propylene glycol (final pH 5.4).

An aqueous solution of sodium phenobarbital without propylene glycol was also prepared in Sorenson buffer (2/15 *M*, pH 8). All preparations were stored in amber glass containers.

Phenobarbital Elixir. A commercially prepared elixir (20 mg/5 ml, Lilly, Lot 9NN12A) was used throughout the experiment.

High-Performance Liquid Chromatographic (HPLC) Assay

One milliliter of sample (emulsion, elixir, or solution) was mixed with methanol (9 ml), vortexed, and centrifuged (International Equipment Company, Needham Heights, Mass.) at 3000 rpm. Mixtures were then syringe filtered using a nylon filter (0.45-μm) membrane (Anspec H3404, P.O. Box 7730, Ann Arbor, Mich.).

Clear filtrate (20 μl) was injected onto the HPLC system, which consisted of a Model 825 pump (Instrument Specialities Company, 4700 Superior Street, Lincoln, Neb.), Model 226 UV (254-nm) detector, and BD-40 recorder (Kipp and Zonen, P.O. Box 507, 2600 AM Delft, Delft, Holland). The column was an ODS-Ultrasphere (Alltech Associates, Inc., Applied Science Labs, 205 Waukegan Road, Deerfield, Ill.) (5 μm, 4.6-mm ID × 15 cm) and the mobile phase was 55% methanol in 1/10 *M* acetic acid (flow, 1.2 ml/min). The retention time of phenobarbital was 3 min. A standard consisting of phenobarbital (4 mg/ml) in methanol was prepared daily. Calculations were based upon the average peak height of sample to the average peak height obtained from injections of standard solution which bracketed the sample injections. To establish linearity a stock solution of phenobarbital (0.5 mg/ml) in methanol was prepared and then diluted further with methanol to give varying concentrations of phenobarbital (0.5, 0.4, 0.2, 0.1, and 0.05 mg/ml). The entire procedure was repeated once.

Linear least-squares was performed on all individual (*r*

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= 0.99) peak heights. The intercept was not significantly different from zero ($P \geq 0.01$).

Recovery studies were performed on the emulsions prepared with and without propylene glycol. Three samples (1 ml) of each dosage form were assayed with duplicate injections. A mixture of 1 ml of emulsion without phenobarbital and 1 ml of phenobarbital (4 mg/ml in methanol) was qs'd to 10 ml with methanol and used as the standard for the emulsion dosage forms. The average recovery for the emulsion with propylene glycol was 102% (±2%); that of the emulsion without propylene glycol was 100% (±1%). A t test (two tailed) was performed on the average peak height for each dosage form and standard. There was no significant difference for either emulsion compared to the standard ($P \geq 0.01$). The peak height of the commercially prepared elixir was 110% (±1%) and significantly different from that of a standard of 0.4 mg/ml phenobarbital in methanol ($P < 0.01$). This may be a reflection of a higher concentration in the elixir than the label claim. Since this study is concerned with the breakdown of phenobarbital over time, all values in the stability study were expressed as percentage remaining.

A phenobarbital emulsion (4 mg/ml, pH 5, Sorenson buffer, 40% propylene glycol) was prepared to determine the precision of the assay. Seven samples of the emulsion were extracted and injected onto the HPLC in triplicate. An ANOVA between extractions was performed, with no significant difference between extractions ($F = 4.21$, $P \geq 0.01$). The coefficient of variation between extractions was 1.4%.

To determine the reproducibility of the emulsion preparation procedure, four emulsions containing propylene glycol were prepared (4 mg phenobarbital/ml). Each emulsion was extracted once and injected four times. No significant difference ($F = 0.84$, $P \geq 0.01$) was observed between emulsions.

Homogeneity of variance was evaluated for each ANOVA using Bartlett's test for multiple sample variances. No significant difference between variances was detected at the 1% level.

RESULTS AND DISCUSSION

A solution of sodium phenobarbital equivalent to 40 mg of free acid was prepared in a pH 8 Sorenson buffer and heated for various times at 98°C to establish if degradation products would interfere with the peak height of the parent compound. Figure 1 illustrates a typical chromatogram obtained. The peak (B) representing phenobarbital (retention time of 3 min) diminishes and other peaks (A and C) appear. The height of A and C increase with time, while C diminishes.

Three preparations of each dosage form (pH 8 solution, propylene glycol solution, and two different emulsions) were used for the stability study. Only one commercially purchased elixir was used throughout the experiment. A fresh standard was prepared daily.

Table I summarizes the average percentage remaining for each of the dosage forms.

The emulsions prepared without propylene glycol showed a rapid decline of phenobarbital concentration with time followed by a period in which the concentration was stable. This initial decline was accompanied by the formation of a precipitate which was not readily redispersed. After 4 weeks the remaining phenobarbital in the preparation appeared stable. The emulsions with propylene glycol, the propylene glycol solution, and the elixir (but not the aqueous solution) all appear to be stable. Assuming a first-order degradation process, a linear least-squares analysis was performed on the log of the percentage remaining as a function of time. This was calculated for each preparation of each dosage form. The average first-order half-lives obtained from each preparation within a dosage form were 693 (±240), 990 (±163), 770 (±27), and 130 (±11) weeks for the propylene glycol emulsion, elixir, propylene glycol solution, and aqueous solution, respectively. With the exception of the aqueous solution, it is not possible to reject the hypothesis that the slopes of the individual percentage remaining versus time plot within each dosage form are equal to zero ($P > 0.01$). This would suggest that the emulsion, elixir, and propylene glycol are all quite stable over the length of the study. For this reason, it is inappropriate to compare the half-lives of these preparations as an indicator of stability relative to one another. This does not negate the fact, however, that all three dosage forms appear to be quite stable.

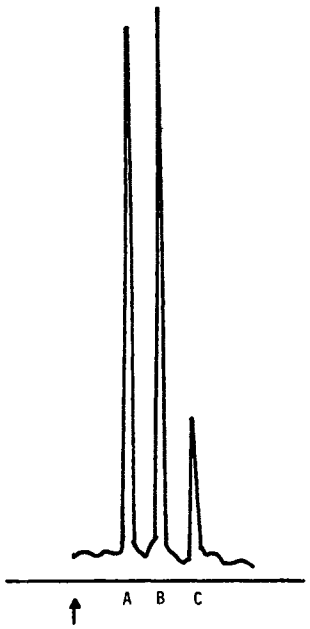

Fig. 1. A representative chromatogram of phenobarbital after heating to facilitate degradation. The arrow indicates the time of injection. Peaks A and C represent breakdown products, while peak B is phenobarbital.

The oil and water phases for the two emulsions were separated by centrifugation and 1 ml of each phase was assayed for phenobarbital. The results are summarized in Table II. A t test indicates no significant differences ($P > 0.01$) between the oil phases of the two emulsions but a difference between the aqueous phases ($P < 0.01$). The emulsion with propylene glycol contains 25% of the total phenobarbital in the oil phase. The total recovered phenobarbital from both phases was 42 mg/10 ml (105% recovery). The emulsion without propylene glycol contains 42% of the total phenobarbital in the oil phase but the total recovered phenobarbital from both phases was 24 mg/10 ml (60% recovery). This

Table I. Average Percentage Remaining Dosage Form

Week	Emulsion[a]	Emulsion[b]	Elixir[c]	Solution[d]	Solution[e]
1	100	100	100	100	100
2	100	95	97	97	94
4	100	66	97	96	95
6	96	67	94	96	95
8	97	69	—	95	93
10	98	68	—	96	91
12	98	68	—	95	90
17	94	—	97	—	—
55	105	—	100	101	77
56	105	—	101	101	76

[a] Emulsion with propylene glycol.
[b] Emulsion without propylene glycol.
[c] Commercially available elixir.
[d] Propylene glycol solution.
[e] Aqueous solution (pH 8).

suggests that the propylene glycol raised the solubility of the phenobarbital in the aqueous phase. The 60% recovery from the emulsion without propylene glycol is due to precipitation of the phenobarbital from the aqueous phase.

In conclusion we have used a rapid HPLC assay to evaluate the stability of phenobarbital in a number of different dosage forms. Using this assay we have demonstrated that phenobarbital is stable in an elixir, an emulsion, and a propylene glycol solution. The stability of the propylene glycol emulsion is attributed both to the solubilization of the drug in the oil phase and to the use of propylene glycol in the aqueous phase. Increasing the phase volume ratio of oil/water should increase the percentage of the drug in the oil phase, as may altering the quantity or nature of the surface active agents. This study also supports the hypothesis that it may be possible to maintain stability of drugs prone to degradation in aqueous solutions by dispersing them in an emulsion as an alternative to an elixir. Our lab is evaluating emulsions of other drugs to determine if the emulsion dosage form can enhance the bioavailability of these drugs.

Table II. Concentration (mg/ml) of Phenobarbital in Emulsion Dosage Forms

	Emulsion A[a]		Emulsion B[b]	
Sample	Oil phase	Water phase	Oil phase	Water phase
1	3.48	4.56	3.48	1.92
2	3.49	4.68	3.76	1.62
3	2.92	4.68	3.52	2.12
Mean	3.30	4.64	3.59	1.88
SD	0.33	0.69	0.15	0.25

[a] Emulsion with propylene glycol.
[b] Emulsion without propylene glycol.

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