Belladonna Alkaloids and Phenobarbital Combination Pharmaceuticals Analysis II: High-Performance Liquid Chromatographic Determination of Phenobarbital

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Abstract □ A high-performance liquid chromatographic separation is described for the analysis of phenobarbital in combination pharmaceutical dosage forms containing belladonna alkaloids. A mobile phase of 0.003 *M* tetramethylammonium chloride in water–methanol (3:2, pH 7.4) was used to separate phenobarbital from belladonna alkaloids on an octadecylsilane column in <7 min. The column effluent was monitored at 240 nm, which resulted in a detection limit of 6 ng of phenobarbital. The method is applicable to elixirs, tablets, and capsules with no preliminary extraction procedure. Data from the application of the method to commercial products is also presented.

Keyphrases □ High-performance liquid chromatography—belladonna alkaloids and phenobarbital combination pharmaceuticals, determination of phenobarbital □ Phenobarbital—belladonna alkaloids, combination pharmaceuticals, high-performance liquid chromatographic determination □ Combination drugs—belladonna alkaloids, high-performance liquid chromatographic determination of phenobarbital

Phenobarbital is present in elixirs, tablets, and capsules with belladonna alkaloids for its sedative effects. It has been previously analyzed by such procedures as extraction with UV detection (1), partition chromatography with UV detection (2), derivative formation with gas chromatography (3–5), normal-phase high-performance liquid chromatography (HPLC) (6, 7), and reversed-phase HPLC (8–10). Since an assay was desired which would work as rapidly and as effectively with elixirs as with tablets and capsules with minimum sample preparation, reversed-phase HPLC was investigated.

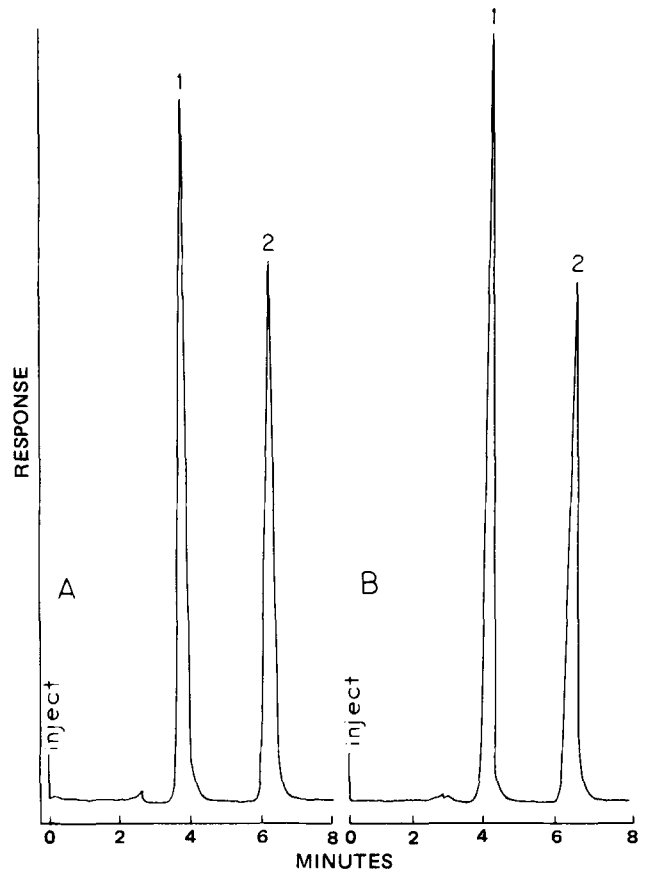

Figure 1—*Typical HPLC chromatograms showing separations of A, the standard solution and B, the sample solution. Key: 1, phenobarbital; 2, guaifenesin (internal standard).*

The HPLC retention properties of phenobarbital at different pH values of the mobile phase using ammonium phosphate and ammonium carbonate buffers was investigated previously (8). It was found that phenobarbital could not be resolved from the solvent front on 37–40-μm commercially packed reversed-phase columns, and that the phenobarbital peak resolution was not improved by variations in either pH or the methanol–water ratio. Others, also investigating the reversed-phase retention properties of phenobarbital at different pH values of the mobile phase (9) found, using sodium phosphate buffers, that retention volumes would change with the pH of the mobile phase on a 10-μm commercially packed reversed-phase column. However, no optimization of analytical conditions was attempted. Reversed-phase HPLC was used also for the analysis of phenobarbital in animal feeds with an unbuffered methanol–water mobile phase (10). However, the mobile phase was selected to minimize animal feed interferences.

A reversed-phase HPLC procedure was developed in this laboratory which is rapid, specific, and stability indicating for the analysis of phenobarbital in the presence of belladonna alkaloids in commercial elixirs, tablets, and capsules. Analytical data for selected commercial products using the proposed method are reported.

EXPERIMENTAL

Apparatus—A liquid chromatograph[1], equipped with a 20-μl loop injector[2], a variable wavelength UV detector[3], and a recorder-integrator[4], was used. A 25 cm × 4-mm i.d. stainless steel column containing octadecylsilane chemically bonded to 5-μm silica[5] was employed.

Reagents—A 0.01 *M* tetramethylammonium chloride solution was prepared by mixing 1.1 g of tetramethylammonium chloride[6] in 1 liter of distilled water. A 0.02 *M* phosphate buffer (pH 7.4) was prepared by dissolving 6.80 g of monobasic potassium phosphate in ~250 ml of distilled water, adjusting to pH 7.4 with ~39 ml of 1 *N* NaOH, and diluting to 1 liter.

Preparation of Internal Standard Solution—A guaifenesin[7] internal standard solution in methanol (3.2 mg/ml) was prepared (Fig. 1).

[1] Constametric I Pump, Laboratory Data Control, Riviera Beach, Fla.
[2] Chromatography Accessory Module Injector, 20-μl loop, Laboratory Data Control, Riviera Beach, Fla.
[3] Spectromonitor II Variable Wavelength UV-Visible Detector, Laboratory Data Control, Riviera Beach, Fla.
[4] HP 3380A Integrator, Hewlett-Packard, Palo Alto, Calif.
[5] Spherisorb octadecylsilane, 5 μm. Prepacked column purchased from Laboratory Data Control, Riviera Beach, Fla.
[6] Aldrich Chemical Co., Milwaukee, Wis.
[7] K&K Laboratories, Plainview, N.Y.

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Table I—Recovery Data for Spiked Sample Determinations of Commercial Formulations for Phenobarbital [a]

Elixirs		Tablets and Capsules	
Formulation [b]	Percent Recovery	Formulation [b]	Percent Recovery
2	100.0	10	99.2
5	98.0	15	101.4
9	99.9	19	101.8
		20	100.8
Mean Recovery	99.3		100.8
SD	1.1		1.1
RSD	±1.1		±1.1
Range	98.0–100.0		99.2–101.8

[a] Portion of sample spiked with standard solution containing phenobarbital and analyzed according to procedure. [b] Numbering corresponds to formulations in Table II.

Preparation of Drug Standard Solution—A phenobarbital[8] standard solution was prepared in absolute methanol (260 μg/ml). Five milliliters of this solution was added to 4.0 ml of guaifenesin internal standard solution and 30 ml of 0.02 *M* phosphate buffer (pH 7.4), cooled to room temperature, and the volume adjusted to 50.0 ml with absolute methanol.

Preparation of Mobile Phase—A mobile phase was prepared by mixing 150 ml of 0.02 *M* phosphate buffer (pH 7.4), 150 ml of 0.01 *M* tetramethylammonium chloride solution, and 200 ml of absolute methanol.

HPLC Conditions—A flow rate of 1.0 ml/min was used with the UV detector set at 240 nm and the detector sensitivity set at 0.04 aufs.

Assay for Phenobarbital in Elixirs—A quantity of elixir equivalent to ~16 mg of phenobarbital was pipeted into a 50-ml volumetric flask. Thirty milliliters of methanol was added to the flask, the contents shaken for 4 min, and made to volume with methanol. About 5 ml of the solution was passed through a 0.5-μm filter. Aliquots of 2.0 ml of the filtrate and 2.0 ml of guaifenesin internal standard solution were combined with 15 ml of 0.02 *M* phosphate buffer (pH 7.4) into a 25-ml volumetric flask. The solution was mixed and made to volume with methanol. A 20-μl portion of the solution was injected into a liquid chromatograph. The peak response was compared with the peak response by peak height or peak area of 20 μl of phenobarbital standard solution. The quantity of phenobarbital in the portion of sample taken was calculated by the formula $(Ru/Rs) \times C$ in which Ru and Rs were the ratios of phenobarbital to guaifenesin peak response for sample (u) and standard (s), respectively, and C was the concentration of phenobarbital in the standard solution.

Assay for Phenobarbital in Tablets and Capsules—Twenty tablets or the contents of 20 capsules were weighed and finely powdered. A portion of sample composite equivalent to ~16 mg of phenobarbital was accurately weighed and transferred to a 50-ml volumetric flask. Five milliliters of distilled water was added and the contents of the flask shaken for 1 min. The procedure for "Assay for Phenobarbital in Elixirs" was then followed beginning with, "Thirty milliliters of methanol was added . . .."

Content Uniformity in Tablets and Capsules—A tablet or the contents of one capsule were quantitatively transferred to a volumetric flask which would yield a concentration of ~0.32 mg/ml of phenobarbital. Five milliliters of distilled water was added and the contents of the flask were mixed for 1 min. The "Assay for Phenobarbital in Elixirs" was then followed beginning with, "Thirty milliliters of methanol was added . . .."

RESULTS AND DISCUSSION

A slightly basic mobile phase (pH 7.4) was used to increase the UV absorptivity of phenobarbital, while avoiding the considerable column degradation normally observed with a mobile phase at a more basic pH. The addition of tetramethylammonium cations to the mobile phase resulted in selective increased retention of anionic compounds (*i.e.*, phenobarbital) through the formation of a tetramethylammonium ion-pair (11). Increased peak broadening with a resulting loss in peak resolution occurred when samples or standards were dissolved in methanol instead of in the mobile phase.

No intefering peaks were detected with 20 min of injection in any of the commercial products analyzed. The belladonna alkaloids did not interfere because of their separate retention times, lower absorptivities, and smaller sample concentrations. The column was found to be stable with daily usage for more than 1 month with no apparent loss in resolution of phenobarbital and guaifenesin internal standard.

[8] USP reference standard.

Table II—HPLC Analysis Results for Commercial Formulations

Formulation	Dosage Form	Percent of Label Claim [a]
1	Elixir	101.9
2	Elixir	102.2
3	Elixir	102.5
4	Elixir	98.8
5	Elixir	99.7
6	Elixir	98.1
7	Elixir	108.6
8	Elixir	101.2
9	Elixir	95.6
10	Tablet	99.0, (100.0) [b]
11	Tablet	104.7, (104.7)
12	Tablet	92.4, (96.9)
13	Tablet	104.6, (102.2)
14	Tablet	100.6, (100.6)
15	Tablet	104.4, (102.1)
16	Tablet	97.5, 100.6 [c], (101.2)
17	Tablet	104.9, 101.9 [c], (100.0)
18	Tablet	96.9, (100.6)
19	Tablet	100.4, 101.2 [c], (101.2)
20	Capsule	106.8, 106.8 [c], (109.2)
21	Capsule	105.0 (101.9)

[a] Label claim range 15–32 mg/unit dose. [b] Values in parentheses are the average of 10 individual tablet assays (content-uniformity). [c] Value obtained by second analyst.

Six replicate injections of a standard solution containing 0.026 mg/ml of phenobarbital gave a coefficient of variation of 0.5% for the ratio of the peak response of phenobarbital to that of internal standard. Linear response was obtained in the 0–0.055 mg/ml range for a series of five phenobarbital standards.

Seven commercial formulations were each spiked with a standard solution of phenobarbital, extracted and assayed, and the percentage of standard recovery calculated. The recovery results and the statistical evaluation of the recovery data are summarized in Table I.

Twenty-one commercial formulations consisting of elixirs, tablets, and capsules were assayed. Tablets and capsules were also analyzed for content uniformity. Results of the content uniformity analyses are summarized in Table II. The average content uniformity values agreed with the composite assay values. Composite assays on Formulations 16, 17, 19, and 20, listed in Table II, were performed by another analyst. These results compared favorably with the original findings.

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