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Application of Postcolumn Ionization in the High-Performance Liquid Chromatographic Analysis of Butabarbital Sodium Elixir

EDITH P. SCOTT

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Abstract □ A sensitive postcolumn ionization high-performance liquid chromatographic (HPLC) method for the quantitative determination of butabarbital sodium in butabarbital sodium elixir is described. The procedure employs a octadecylsilane column chemically bonded to porous silica microparticles. The mobile phase is a mixture of methanol and water (typically 35:65), adjusted to provide separation of butabarbital from two degradation compounds and other formulation ingredients. A buffer (pH 10) added between the column and detector provides for the primary ionization of the barbiturate necessary for optimum UV-detector sensitivity at ~240 nm. Determinations are made using the sodium salt; thus the need for extraction of the free base is eliminated. The procedure is linear over the 0.3–0.9-mg/ml concentration range of butabarbital sodium. Reproducibility values for 10 injections of a single reference standard range from 100.2 to 100.8% of theoretical with a mean of 100.5% and a coefficient of variation of 0.23%. An interlaboratory precision study for blind duplicates of one simulated product formulation and two commercial elixers produced coefficients of variation of 1.4, 1.3, and 1.1%, respectively. Recovery determinations for the drug in simulated product formulations ranged from 98.4 to 99.0%, intralaboratory, and 97.7 to 102.2%, interlaboratory. The HPLC procedure is stability indicating with respect to two decomposition products.

Keyphrases □ Butabarbital sodium—application of postcolumn ionization in higher-performance liquid chromatographic analysis, elixir □ Ionization—postcolumn, application in high-performance liquid chromatographic analysis of butabarbital sodium elixir □ High-performance liquid chromatography—application of postcolumn ionization, analysis of butabarbital sodium elixir

The chromaphoric characteristics of barbiturates establish them as prime compounds for high-performance liquid chromatographic (HPLC) analysis using a combination of reverse-phase chromatography and postcolumn ionization with a pH 10 buffer. Clark and Chan (1) have reported the advantages of combining conventional reverse-phase chromatography and postcolumn ionization into a single system. In this study, their analytical approach has been successfully employed in the assay of butabarbital sodium elixir. The procedure eliminates the need for extraction of butabarbital free acid. No sample cleanup was required because a suitable chromatographic system was found that would resolve the phenobarbital internal standard, butabarbital, placebo ingredients, and one decomposition product, capuride.

Table I—Typical HPLC Standard Curve Data [a] for Butabarbital Sodium

Butabarbital Sodium Added, mg/50 ml	Butabarbital Sodium, Found, mg/50 ml	Percent of Theoretical
16.73	16.54	98.9
23.24	23.19	99.8
30.36	30.27	99.7
38.00	38.00	100.0
46.00	45.82	99.6

[a] Correlation coefficient = 0.9999.

EXPERIMENTAL

Apparatus—The liquid chromatograph[1] consisted of a solvent pump with flow controller; an injector with flowing-stream, valve-controlled

[1] Waters Liquid Chromatograph; Model 6000-A Solvent Delivery System, Model 720 System Controller, Model U6K Injector, Model 440 Absorbance Detector with 254 nm filter, Model 730 Data Module; Waters Associates, Milford, Mass.

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Table II—HPLC Assay Results for Commercial Butabarbital Sodium Elixir and Recovery Data for Corresponding Product Control [a]

	Manufacturer			
	A	B	C	D
Assay of drug in commercial products				
Butabarbital sodium found (mg/5 ml)	29.2	32.9	29.0	31.7
Butabarbital sodium declared (mg/5 ml)	30.0	33.3	30.0	33.0
Percent of declared	97.3	98.8	96.7	96.1
Recovery for drug in authentic formulations				
Butabarbital sodium found (mg/5 ml)	30.48	31.26	30.09	30.87
Butabarbital sodium added (mg/5 ml)	30.92	31.59	30.47	31.36
Recovery, %	98.6	99.0	98.8	98.4

[a] Small batch of commercial product, accurately prepared in the laboratory according to the manufacturer's master formulation.

specimen loop; a UV-detector capable of monitoring absorbance at ~240 nm; a digital integrator–recorder; and a 10-μm micropellicular octadecylsilane column[2]. To achieve postcolumn ionization, a second solvent pump[3] was connected to the HPLC apparatus by means of a 1.58 mm union tee[4] installed in the line between the column and the detector.

Reagents—The methanol[5] was distilled in glass. The boric acid[6], potassium chloride[7], and sodium hydroxide[5] were ACS grade. Phenobarbital[8] was USP grade; the butabarbital[9] was USP reference standard[9].

HPLC Operating Conditions—The mobile phase consisted of methanol–distilled water (35:65), and the flow rate was 1.5 ml/min. The ionization solvent was a borate buffer (pH 10.0 ± 0.05) prepared by mixing 250 ml of 0.2 *M* boric acid, 250 ml of 0.2 *M* potassium chloride, and 220 ml of 0.2 *M* sodium hydroxide and diluting to 1 liter with distilled water. The buffer was pumped into the mobile phase at a flow rate of 0.1 ml/min. The UV detector was operated with a 254-nm filter and a sensitivity setting of 0.1 AUFS. Injection volume for sample and standard solutions was ~7.5 μl; injections were made in triplicate. A resolution value, R[10], of ≮1.5 was established to evaluate system suitability.

Preparation of Solutions—A solution of phenobarbital in methanol was prepared at a concentration of ~3 mg/ml for the internal standard. Aliquots of the internal standard solution and butabarbital sodium elixir were combined to produce a mixed solution containing ~0.3 mg/ml of phenobarbital and 0.7 mg/ml of butabarbital sodium in methanol. Butabarbital and an aliquot of the internal standard solution were combined to produce a mixed solution (standard) containing ~0.3 mg/ml of phenobarbital and 0.6 mg/ml of butabarbital in methanol.

Chromatographic Separation and Analysis—A printer–plotter–integrator[11] which automatically integrates chromatographic peaks and calculates results was employed in the liquid chromatograph apparatus. The data processor calculated peak areas and response factors resulting from injection of a standard solution and stored the data in a calibration table. Similarly, the data processor calculated areas and response factors for injections of sample solutions and compared sample data with standard data in the calibration table for calculation of sample amounts. Phenobarbital elution time was ~9 min, and butabarbital elution was ~13 min (Fig. 1).

RESULTS AND DISCUSSION

The HPLC procedure described herein was subjected to validation studies for linearity, precision, and accuracy. Linearity was determined at five concentrations which would be equivalent to butabarbital sodium elixir products with assay values ranging from 50 to 150% of declared butabarbital sodium. Assay results ranged from 98.9 to 100.0% of theoretical, with a correlation coefficient of 0.9999 (Table I). Reproducibility for 10 injections of a butabarbital sodium standard solution ranged from 100.2 to 100.8% of theoretical, with a mean of 100.5% and a coefficient of variation of 0.23% (Table III).

Four brands of butabarbital sodium elixir were assayed by the HPLC procedure. Recoveries were determined using product controls prepared for these four brands of elixir. A product control is defined here as a small batch of commercial product accurately prepared in the laboratory according to the manufacturer's master formulation. Assay results and recoveries for the corresponding product controls are shown in Table II.

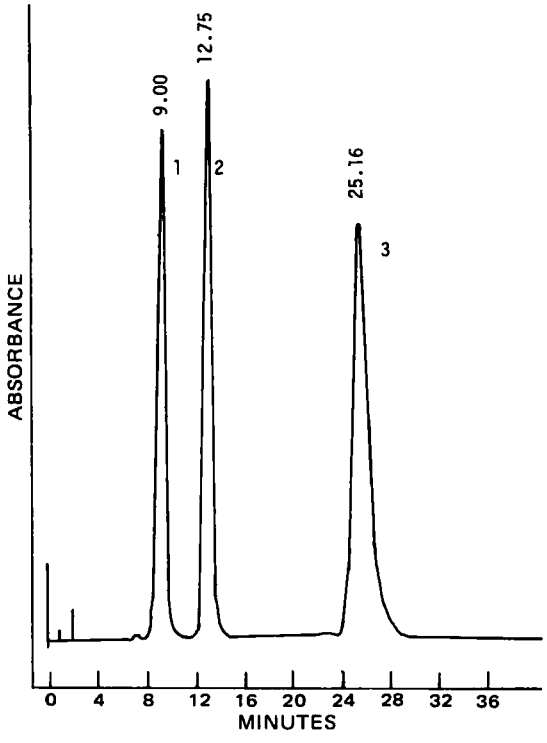


Figure 1—*HPLC response for (1) phenobarbital, (2) butabarbital, and (3) capuride. The column was μBondapak C_{18}; the mobile phase was methanol–distilled water (35:65); ionization solvent was borate buffer (pH 10).*

An interlaboratory collaborative study of the procedure was conducted using blind duplicate samples of one simulated product formulation and

Table III—Precision Data for Multiple HPLC Injections of Butabarbital Sodium [a]

Butabarbital Sodium Found by HPLC, mg/50 ml	Percent of Theoretical
34.85	100.2
35.03	100.7
35.00	100.7
35.04	100.8
34.92	100.4
34.95	100.5
34.86	100.2
34.89	100.3
34.98	100.6
34.93	100.5
Mean	100.5
SD	0.23
CV	0.23

[a] Butabarbital sodium added: 34.77 mg/50 ml.

[2] μBondapak C_{18}, 3.9 mm × 30 cm, Waters Associates, Milford, Mass.
[3] Model 6000-A Solvent Delivery System; Waters Associates, Milford, Mass.
[4] Swagelok Union Tee, SS-100-3, Crawford Fitting Co., Solon, Ohio.
[5] ACS grade, Burdick and Jackson Laboratories, Inc., Muskegon, Mich.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Fisher Scientific Co., Fair Lawn, N.J.
[8] ACS grade; City Chemical Corp., New York, N.Y.
[9] USP grade; U.S. Pharmacopeial Convention, Inc., Rockville, Md.
[10] USP Reference standard; U.S. Pharmacopeia XX, (621) Chromatography, Glossary of Symbols, p. 946.
[11] Waters Model 730 Data Module.

Table IV—Interlaboratory Analysis of Blind Duplicate Samples of Butabarbital Sodium Elixir

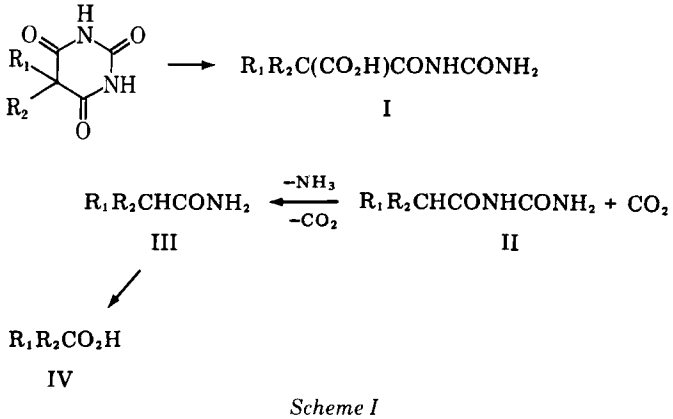
Laboratory	Sample A[a] Recovered, %		Sample B[b] Percent of Declared		Sample C[c] Percent of Declared	
1	101.2	100.1	98.7	98.0	97.0	96.7
2	99.1	98.0	95.3	95.7	95.2	95.8
3	100.8	100.5	97.7	97.7	94.9	95.2
4	100.8	102.2	97.0	97.0	97.3	97.9
5	99.1	97.7	94.7	95.7	94.9	96.4
6	101.2	100.5	98.3	97.3	96.1	97.3
Mean	100.1%		96.9		96.2%	
SD	±1.36		±1.28		±1.03	
CV	1.4%		1.3%		1.1%	

[a] Simulated product formulation with 28.36 mg/5 ml of butabarbital sodium added. [b] Commercial product with 30 mg of butabarbital sodium per 5 ml declared. [c] Commercial product with 33.3 mg of butabarbital sodium per 5 ml declared.

two commercial elixirs. Coefficients of variation were 1.4, 1.3, and 1.1%, respectively, for the three samples. Recovery values for the authentic sample ranged from 97.7 to 102.2%. Results of interlaboratory testing are presented in Table IV.

A placebo was prepared for each of the four butabarbital sodium elixir products analyzed. None of the placebos interfered with the assay (Fig. 2). Solutions of butabarbital sodium and butabarbital in methanol were found to be stable over a prolonged period in laboratory fluorescent light and laboratory temperature (generally 20–25°). Stability determinations were made after 11 and 56 days of exposure time.

Limited data on HPLC response for decomposition products was obtained. No commercial source for butabarbital decomposition compounds was available; thus, an attempt was made to prepare the compounds through thermal degradation of butabarbital sodium in this laboratory. Breakdown products due to hydrolysis of barbiturates (Scheme I) have been established (2–5).



Scheme I

Two approaches to thermal decomposition were employed. With the Watson and Pernarowski procedure (5) only one compound, capuride (II), was obtained in sufficient quantity for analytical testing. Thermal decomposition using a pressure bottle (6) was also attempted to isolate additional decomposition products. This procedure also produced capuride and, additionally, a compound which was identified as valnoctamide (III). Both the capuride and valnoctamide which were produced were identified by means of melting point, IR spectrophotometry, and mass spectroscopy.

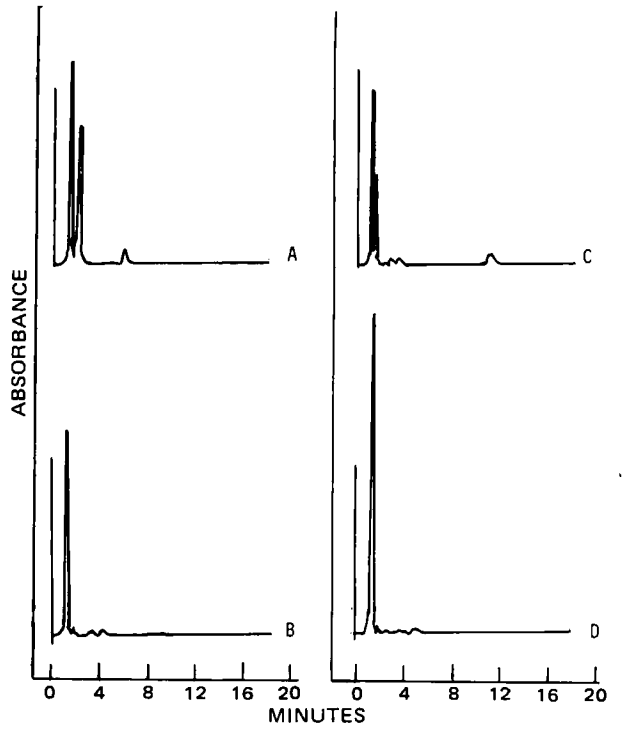


Figure 2—*HPLC responses for placebo ingredients from manufacturers A–D (see Table II).*

Capuride eluted from the HPLC column at 25.16 min. (Fig. 1). An injection of ~10 μg produced a pen deflection of ~1.5 cm at 0.02 AUFS. No HPLC response was produced for a 35-μg injection of valnoctamide at 0.02 AUFS.

Chromatograms of phenobarbital, butabarbital, and product placebos from injections made on six octadecylsilane columns representing five commercial sources and one in-laboratory preparation were evaluated. Columns that met the system suitability criterion gave satisfactory resolution of phenobarbital, butabarbital, and placebo ingredients.

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