High-Performance Liquid Chromatographic Method for the Determination of Esmolol Hydrochloride

YING-CHI LEE, DAVID MICHAEL BAASKE ˣ, and ABU S. ALAM

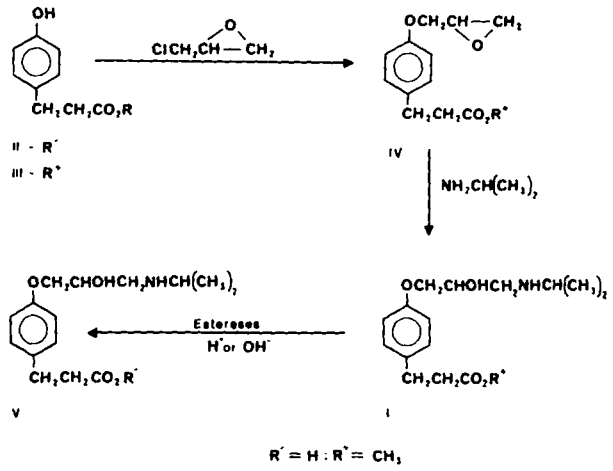
Received September 14, 1983, from the *Pharmaceutical Development Department, Research and Development Division, American Critical Care, McGaw Park, IL 60085.* Accepted for publication January 13, 1984.

Abstract □ A rapid high-performance liquid chromatographic method for the determination of esmolol hydrochloride, a new ultra-short-acting beta blocker, is described. The stability-indicating nature of the method was demonstrated by resolving esmolol from synthetic intermediates, potential impurities, and the product of decomposition. Reverse-phase liquid chromatography was performed with a microparticulate (10-μm) cyano-bonded silica-packed column, a fixed-wavelength UV absorbance detector (λ = 280 nm), and a mobile phase of acetonitrile–0.005 M sodium acetate–acetic acid (15:84:1) pumped at 2 mL/min. The internal standard was 2-*p*-chlorophenyl-2-methylpropanol. A percent *RSD* of <1.7% and an accuracy (100% − mean error) of >98.6% were achieved over the concentration range studied (100–500 μg/mL), with correlation coefficients >0.9996.

Keyphrases □ HPLC—determination of esmolol hydrochloride □ Esmolol hydrochloride—HPLC

Esmolol hydrochloride (methyl 3[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate hydrochloride; I) is the first of a new class of beta blockers, known as ultra-short-acting beta blockers, to enter clinical trials. The ultra-short-acting beta blockers were designed to extend the usefulness, safety, and efficacy of beta blockers in critical cardiac therapy through controlled and titratable intravenous therapy (1). This was accomplished by designing chemical instability into the molecule. The ester functionality of the molecule is susceptible to cleavage by the nonspecific serum esterases to the free acid 3[4-[2-hydroxy-3-[(2-methylethyl)amino]-propoxy]phenyl]propionic acid.

Esmolol hydrochloride was synthesized (2) from 3-(*p*-hydroxyphenyl)propionic acid (II) *via* a four-step process (Scheme I). In this report the development and validation of a high-performance liquid chromatographic (HPLC) method for the quantitation of esmolol in the presence of synthetic intermediates (II–IV) and free acid (V) is described.



Scheme I

EXPERIMENTAL SECTION

Materials—Compounds I, III, IV, V, and VI were synthesized in this laboratory. 2-(*p*-Chloro-phenyl)-2-methyl propanol[1], compound II, glacial acetic acid[2], and sodium acetate[2] were used as received. Glass-distilled acetonitrile and methanol were used for all procedures[3]. Purified water[4] was used throughout.

Chromatography—A liquid chromatographic system (3), equipped with a fixed-wavelength UV absorbance detector[5] at 280 nm, an on-line data system[6], and a column (30 cm × 3.9 mm) packed with cyano-bonded silica (10-μm)[7], was used. Sample injections were 50 μL. Mobile phase was prepared fresh daily by thoroughly mixing 150 mL of acetonitrile, 10 mL of glacial acetic acid, and 840 mL of sodium acetate trihydrate (0.068%, w/v) buffer which was filtered through a 0.5-μm filter[8] prior to use. A constant flow rate of 2 mL/min yielded a pressure of <2000 psi.

Standard concentrations of 500, 400, 300, 200, 100, and 0 μg/mL were prepared in quadruplicate. To 1.0 mL of each standard or sample was added 750 μL of the internal standard 2-(*p*-chlorophenyl)-2-methyl propanol (4 mg/mL) in methanol–water (50:50). Peak areas were measured, and the ratios for esmolol–internal standard were calculated with the data system, yielding a calibration curve. Samples were prepared in water at 500 μg/mL.

Specificity of the Method—Esmolol hydrochloride (50 mg) was placed in each of four 100-mL volumetric flasks. Into each flask was added 75 mL of either water, 1 M HCl, 1 M NaOH, or 30% hydrogen peroxide. The flasks were gently boiled for 1 h. After cooling, the pH was adjusted to 4 with concentrated

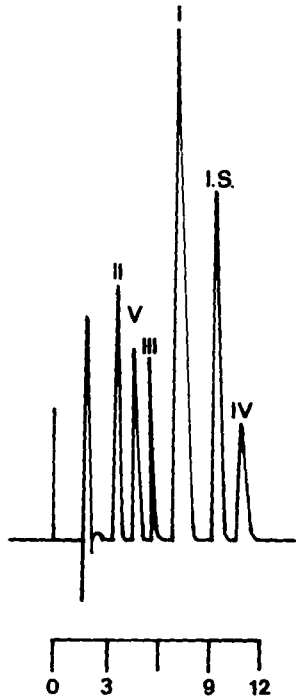


Figure 1—*Typical chromatogram showing the resolution of I from the synthetic intermediates (II–IV) and the anticipated breakdown product (V). IS, internal standard.*

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Analytical reagent; Mallinckrodt, St. Louis, Mo.
[3] Burdick & Jackson Laboratories, Muskegon, Mich., or J. T Baker Chemical Co., Phillipsburg, N.J.
[4] Milli-Q Water Purification System; Millipore Corp., Bedford, Mass.
[5] Model LC-15; Perkin-Elmer Corp., Norwalk, Conn., or model 440; Waters Associates, Milford, Mass.
[6] HP-3354; Hewlett-Packard, Avondale, Pa.
[7] μ-Bondapak CN; Waters Associates.
[8] Millipore Corp.

0022-3549/84/1100-1660$01.00/0

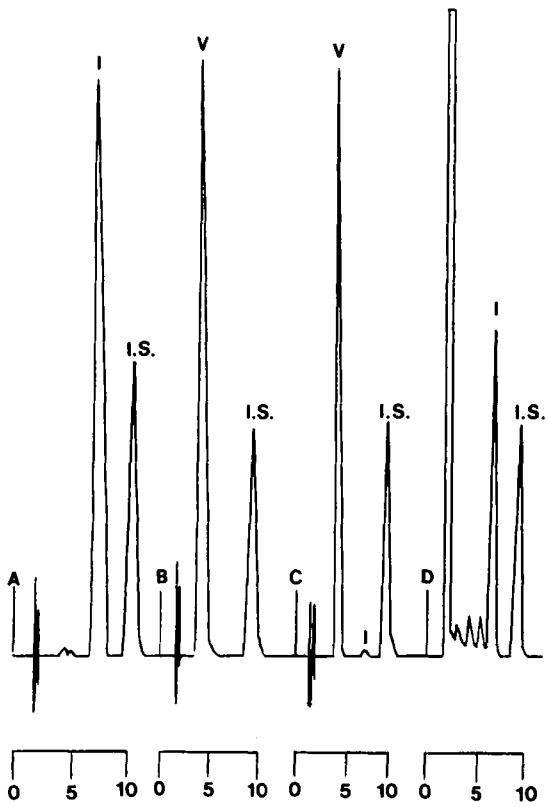


Figure 2—*Chromatogram of esmolol hydrochloride solutions boiled for 1 h. Key: (A) water, pH 5.5; (B) 1 M NaOH; (C) 1 M HCl; (D) 30% H_2O_2.*

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).

REFERENCES

(1) J. Zaroslinski, R. J. Borgman, J. P. O'Donnell, W. G. Anderson, P. W. Erhardt, S.-T. Kam, R. D. Reynolds, R. J. Lee, and R. J. Gorczynski, *Life Sci.*, **31**, 899 (1982).

(2) P. W. Erhardt, C. M. Woo, W. G. Anderson, and R. J. Gorczynski, *J. Med. Chem.*, **25**, 1408 (1982).

(3) D. M. Baaske, N. N. Karnatz, and J. E. Carter, *J. Pharm. Sci.*, **72**, 194 (1983).

ACKNOWLEDGMENTS

The authors thank Dr. Paul W. Erhardt and Mr. Chi M. Woo for the synthetic products cited and Ms. Lori Coomans for assistance in the preparation of this manuscript.

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

V. DAS GUPTA

Received October 18, 1983, from the *Department of Pharmaceutics, University of Houston, Houston, TX 77030.* Accepted for publication January 19, 1984.

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

0022-3549/84/1100-1661$01.00/0