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Note

High-performance liquid chromatographic method for the determination of esmolol hydrochloride in solutions and parenteral formulations

NANCY N. KARNATZ*, DAVID M. BAASKE, DALE E. HERBRANSON and MARK S. ELIASON
Department of Pharmaceutical Development, American Critical Care, 1600 Waukegan Road, McGaw Park, IL 60085 (U.S.A.)
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Esmolol hydrochloride (Brevibloc®, American Critical Care) is an ultra-short acting beta-blocking agent. The ultra-short action of esmolol hydrochloride (I) is due to a built-in chemical instability achieved through the incorporation of an ester linkage within the molecule. Thus, normal chemical degradation can be expected to proceed through hydrolysis to yield 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]-phenyl}propionic acid (II).

A method for active drug substance of I which resolves esmolol from four synthetic intermediate compounds has previously been reported[1].

The previously reported method was developed to resolve the intermediates and was, therefore, not particularly sensitive for esmolol. In order to resolve all peaks potentially present in the active drug substance, consessisons had to be made, including sensitivity. In the method reported here, much greater sensitivity for esmolol was obtained. It is also more rugged, and is suitable for routine use. It requires analysis time of less than 15 min per sample. This report details the development of the assay.

EXPERIMENTAL

Materials

Esmolol hydrochloride (I), 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl} propionic acid hydrochloride (II) and the ethyl ester analogue of (I), ethyl 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl} propionate hydrochloride (III), were synthesized in-house. HPLC-grade potassium phosphate monobasic (Fisher Scientific, Fair Lawn, NJ, U.S.A.), glass-distilled acetonitrile and methanol (J. T. Baker, Phillipsburg, NJ, U.S.A.) were used for all high-performance liquid chromatographic (HPLC) procedures. Water purified by a Milli-Q Water Purification System (Millipore, Bedford, MA, U.S.A.) was used throughout the procedure. *o*-Chlorobenzyl alcohol (Aldrich, Milwaukee, WI, U.S.A.), ethanol (95%, v/v) (U.S. Ind. Chem., Tuscola, IL, U.S.A.), propylene glycol (Union Carbide, Danbury, CT, U.S.A.), sodium acetate, dextrose, benzalkonium chloride, sodium chloride, diethylether, 1-butanol, acetic acid, chloroform, and dichloromethane (Mallinckrodt, St. Louis, MO, U.S.A.) were used as received. Pre-coated silica G-25 UV 254 thin-layer

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chromatography (TLC) plates (Brinkman, Westbury, NY, U.S.A.) were used for all TLC experiments.

Instrumentation

The liquid chromatographic (LC) system consisted of a Series 2 solvent delivery system, an Model 420 automatic sampler, a Model LC-55 UV absorbance detector (all Perkin-Elmer, Norwalk, CT, U.S.A.), a Model 555 (Linear Instruments, Fair Lawn, NJ, U.S.A.) 10-mV recorder, and an HP-3356 (Hewlett-Packard, Avondale, PA, U.S.A.) on-line data system. A 30 cm × 3.9 mm I.D. stainless-steel column packed with octadecylsilane (10 μm) (μBondapak C_{18}, Waters Assoc., Milford, MA, U.S.A.), a detector wavelength of 200 nm, and a chart speed of 20 cm/h were employed. Sample injections of 100 μl were used. A second selectable fixed-wavelength UV absorbance detector (Model 160, Beckman, Berkeley, CA, U.S.A.) set at a wavelength of 214 nm was used in series with the first variable-wavelength UV absorbance detector (Perkin-Elmer) to evaluate two wavelengths simultaneously.

Specificity

Samples of I were prepared in *ca.* 0.1 *M* hydrochloric acid, 0.1 *M* dibasic sodium phosphate and 0.1 *M* tribasic sodium phosphate and stored at room temperature. Two other esmolol · HCl solutions were prepared in 0.5 *M* sodium thiosulfate and in 95% ethanol, and placed in a 75°C oven for 30 days. A final aqueous solution of I was constantly aerated. The excipients, sodium chloride, dextrose, alcohol, propylene glycol, sodium acetate and benzalkonium chloride, were also tested for interference with the method. The specificity of the method was additionally demonstrated by examining the retention behavior of the ethyl analogue of esmolol. Samples of I were examined following forced degradation and during challenge studies.

TLC

All samples, except the pH degradation samples, were analyzed by four different TLC systems (Table I).

Mobile phase

Approximately 1 l of mobile phase was prepared fresh daily by dissolving 3.0 g of potassium phosphate (monobasic) in 650 ml of water. After dissolution of the potassium phosphate, 150 ml of acetonitrile and 200 ml of methanol were added. The mobile phase was thoroughly mixed and filtered through a 0.5-μm filter (Millipore, Bedford, MA, U.S.A.) prior to use. A constant flow-rate of 2 ml/min yielded a pressure of approximately 2000 p.s.i.

TABLE I

THIN-LAYER CHROMATOGRAPHY DEVELOPING SYSTEM

Mobile phase	*Solvent composition*
1	Diethyl ether–ethanol (7:3)
2	Butanol–acetic acid–water (41:1:5) (organic phase)
3	Chloroform–methanol (1:1)
4	Dichloromethane–diethyl ether–methanol (1:1:1)

Sample and standard preparation

A stock standard of I in methanol (25%) and propylene glycol (25%) was prepared to yield a concentration of 0.5 mg/ml. Standard concentrations of 50, 40, 30, 20, 10, and 0 μg/ml were prepared by appropriate dilution with water. Samples were prepared by dilution with water to yield a concentration of 40 μg/ml. Volumes of 5 ml of *o*-chlorobenzyl alcohol (0.2 mg/ml), the internal standard, were pipetted directly into each of the 50-ml flasks containing either sample or standard before they were brought to volume with water. Each standard or sample was chromatographed and peak areas were measured for esmolol and the internal standard by the data system and the peak heights were measured manually. The peak area and peak height ratios for esmolol and the internal standard were then plotted *versus* the esmolol · HCl concentration to yield calibration curves.

RESULTS AND DISCUSSION

Once an apparently suitable method had been developed (Fig. 1), the effects

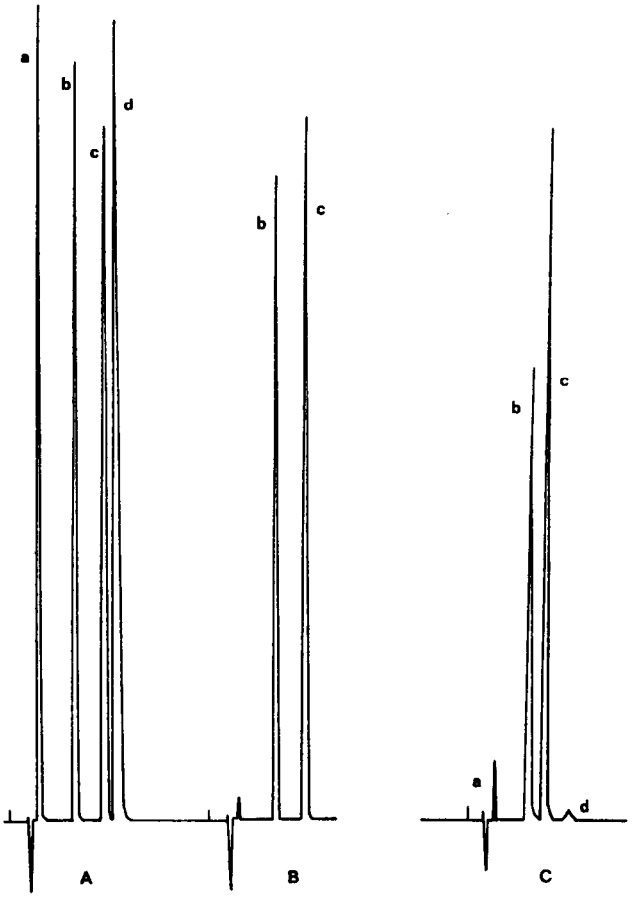

Fig. 1. HPLC chromatograms of esmolol · HCl. (A) Mixture of standards of I, II and III, (B) esmolol · HCl commercial sample, (C) partially degraded esmolol sample in ethanol, 75°C. Peaks: a = II; b = I; c = *o*-chlorobenzyl alcohol (internal standard); d = III.

of small changes in the mobile phase composition were explored. The retention times of esmolol and its analogues were monitored as the concentration of the monobasic potassium phosphate was varied. When the buffer was excluded, all the compounds were completely retained by the column. A stepwise increase in the concentration of monobasic potassium phosphate resulted in a slight decrease in retention for I and III. The free acid (II) was unaffected. These results indicate that the buffer concentration is not a significant factor in retention but it must be present in order for elution to occur.

While holding the buffer concentration constant, the pH of the aqueous phase was modified to observe the effect on retention time. Negligible change in retention time was noted.

The effect of the relative amounts of methanol and acetonitrile was also examined. Incremental changes were made by decreasing the methanol percent by 5% and increasing the acetonitrile by 5%. The composition chosen was methanol–acetonitrile (20:15) because it resulted in optimum peak resolution, symmetry and retention times.

Degradation studies

The 0.5 *M* sodium thiosulfate sample stored at 75°C showed obvious degradation, with an increase in the levels of II with a corresponding decrease in I. The aeration study examined the stability of esmolol · HCl under mildly oxidative conditions. After eight months of testing there was only a slight decrease in potency, revealing that oxidation is not a major degradation pathway for esmolol. These results obtained by HPLC, were confirmed by TLC. Esmolol samples in 95% ethanol at 75°C not only degraded to II, but also were converted to a small extent to III through a transesterification reaction.

The 0.1 *M* hydrochloric acid sample (pH 1.43) completely degraded to II within one week. The 0.1 *M* tribasic sodium phosphate sample (pH 11.5) completely degraded to II within one hour and the less basic sample, 0.1 *M* dibasic sodium phosphate (pH 8.38), was still not completely degraded to II after several months.

In all cases, except ethanol, at 75°C, the only degradation product observed was II.

Selectivity

Sodium chloride (0.9%) injection, and dextrose (5%) (McGaw Labs., Irvine, CA, U.S.A.) injection from large volume parenteral (LVP) solutions were tested for possible interferences. In both cases no peaks were observed at the retention times of interest.

Method statistics and comparison

Three separate analysts, using three separate HPLC systems on three separate days analyzing six ampules of the same lot, achieved an accuracy greater than 97.8% with a standard deviation of less than or equal to 1.08% (see Table II). All standard curves yielded a correlation coefficient for both peak height and peak area measurements of greater than 0.998.

With the procedure as stated, the limit of detection was approximated at 40 ng by visual determination and the limit of quantitation was approximated at 5 μg/ml.

TABLE II

PRECISION DATA — SIX AMPULES OF THE SAME LOT ANALYZED BY THREE ANALYSTS ON SEPARATE DAYS

Reported as percent of labelled amount

	Analyst 1	*Analyst 2*	*Analyst 3*
A	99.3	99.9	98.7
	100.0	98.8	97.5
	100.2	99.6	98.5
B	101.3	100.4	97.7
	101.5	100.7	96.7
	101.0	—*	97.7
C	101.1	—*	96.3
	99.9	98.4	97.6
	101.1	100.6	98.2
D	101.2	99.0	98.6
	100.7	99.1	96.1
	101.2	100.5	99.8
E	99.8	99.8	98.6
	100.2	100.6	96.1
	99.4	101.2	98.9
F	98.8	100.5	98.2
	100.3	99.2	94.4**
	100.0	100.9	99.0
Mean	100.4	99.9	97.9
Standard deviation	0.85	0.84870	1.08

* Sample omitted due to an autosampler malfunction.
** Outlier rejection based on using the Q-test[2].

The method was demonstrated to be suitable at both 200 and 214 nm although most of the work was done at 200 nm. Two sets of esmolol · HCl standard curves were analyzed at both 200 and 214 nm. The correlation coefficient at 214 nm for the standard curves analyzed by peak height were greater than 0.999, those analyzed by the peak area method were 1.000. A paired t-test showed that the results obtained at both wavelengths ($t = 1.15$) were statistically equivalent.

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