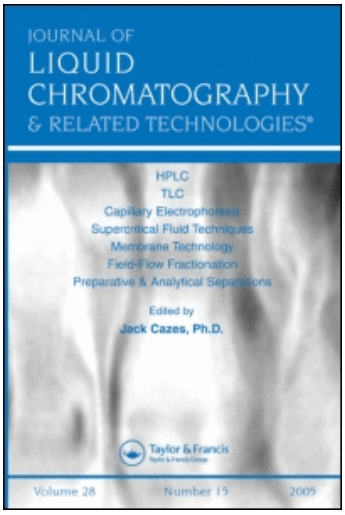

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EFFECT OF TEMPERATURE, EFFECTIVE CAPILLARY LENGTH, AND APPLIED VOLTAGE ON THE MIGRATION OF NINE β-BLOCKERS IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

Study was made on the effect of temperature (25-40 °C), effective capillary length (52-92 cm), and applied voltage (21-29 kV) on the micellar electrokinetic capillary chromatography (MEKC) separations of nine β-blockers and on the electroosmotic breakthrough time. Migration times decreased with increasing temperature and applied voltage and increased with the length of the separation capillary.

INTRODUCTION

Beta-blockers are chemically derived from the adrenergic agonist isoprenaline. Initially, research was directed toward the synthesis of a specific adrenoreceptor antagonist to be used in the treatment of angina pectoris. Propranolol was the first β-blocker to be formally approved for clinical use and

still today is one of the most frequently prescribed drugs in clinical practice (1,2). The several pharmacological properties of propranolol encouraged the subsequent development of a whole series of drugs, now about 50 in number and known as the β-blockers (3). While the basic chemistry depends on the aryloxypropanolamine structure, the nature of the aromatic group or N-terminal side-chain provides the structural differences and the variability of pharmacological action.

The β-blockers have been described as one of the most exciting developments in pharmacology and therapy in recent years (4); there are few groups of drugs that have contributed so broadly to medicine and clinical science (5). Initially applied in the treatment of angina pectoris and cardiac arrhythmias (5), they are now being used as well to control systemic blood pressure and relieve intraocular pressure (6).

The chromatographic separation of β-blockers was first described by Ervik in 1969 (7). Early methods relied on gas chromatography (GC). However, the requirement that the drugs be derivatized to facilitate separation and detection added to the complexity of the technique. Liquid chromatography (LC), much improved since the 1970s, now offers a simpler, more flexible approach (8,9), but, even so, investigators have seldom done more than demonstrate the selectivity of the method. There have also been a number of studies on bioanalysis, but here, too, screening is for a single compound, with another β-blocker used as internal standard (10). Almost all the GC and LC methods

offer screening for just one compound at a time, on the assumption that two β-blockers are unlikely to be taken together.

We recently reported the determination of ten β-blockers simultaneously by micellar electrokinetic capillary chromatography (MEKC) (11). The MEKC method is a development from capillary zone electrophoresis, with one important advantage: because the electrophoretic media are micellar solutions where the surfactant concentration is greater than the critical micelle concentration, neutral and charged molecules can be separated at the same time (12-14).

The normal procedure in MEKC method development is to optimize buffer and surfactant concentrations. Usually the pH of the buffer solution is carefully selected as well. In addition, it is important to optimize the instrumental parameters of MEKC: viz. the separation temperature, the applied voltage, and the effective length of capillary. For example, the temperature affects the CMC of the surfactant (15), and the applied voltage and the length of the capillary affect the migration times of solutes and the selectivity and resolution of the analysis.

In this study, corrected migration times (t'_r) calculated by a well-known formula ($t'_r = t_r - t_0$) were used to describe the migration of the β-blockers. The electroosmotic breakthrough time (t_0) for each run was measured with methanol. Lacking a suitable marker for CTAB micelles, we were unable to calculate capacity factors.

EXPERIMENTAL

Apparatus

A Beckman P/ACE System 2000 Capillary Electrophoresis system was run with P/ACE 2000 series software (Beckman Instruments, INC., Palo Alto, CA, U.S.A.) in an Olivetti M 290 microcomputer (Ing. C. Olivetti & C., S.p.A., Ivrea, Italy). The data were also collected with an HP 3396A integrator (Hewlett-Packard Co., Avondale, U.S.A.). The effect of temperature on MEKC analyses was studied in a 520 mm x 0.050 mm I.D. fused silica capillary tube (Lee Scientific, Salt Lake City, U.S.A.) and the effect of the applied voltage in a 820 mm x 0.075 mm I.D. fused silica capillary tube (Millipore Corporation, Waters Chromatography Division, Milford, MA, U.S.A.). The lengths of fused silica capillary tubes were 920 mm, 820 mm, 720 mm, 620 mm, and 520 mm, with 0.075 mm I.D. (Millipore Corporation). UV detection was at wavelength 214 nm. All experiments were carried out at temperatures from 25 to 40 °C (0.1 °C). Injections were made in high pressure mode for 5 seconds and the running negative voltage at the injector end of the capillary ranged from 21 to 29 kV.

Materials

The β-blockers were acebutolol hydrochloride, alprenolol hydrochloride, atenolol, nadolol, oxprenolol hydrochloride, pindolol, (S)-(-)-propranolol hydrochloride, sotalol hydrochloride, and timolol maleate, all from Sigma Chemical Co. (St. Louis, MO, U.S.A). Acetic acid, sodium acetate, sodium dihydrogenphosphate monohydrate, disodium hydrogenphosphate dihydrate, and N-cetyl-N,N,N-trimethylammonium bromide (CTAB) were from E. Merck

(Darmstadt, GER) and were used as received. Other reagents used in the development of the method were of analytical grade and were used without further purification. Distilled water was purified through a Water-I system from Gelman Sciences Inc. (Ann Arbor., Michigan, U.S.A.). All the micellar buffer solutions were filtered through 0.45 μm membrane filters (Millipore, Molsheim, France) and degassed before use. Samples and other solutions were filtered through Millex filters of 0.5 μm pore size from Nihon Millipore (Kogyo K.K. Yonezawa, Japan).

Buffer solutions

The pH of the buffer solutions was adjusted using a Jenway 3030 pH meter and electrode (Jenway Ltd., Felsted, England). The effect of the temperature was studied under the following conditions: 0.125 M acetate buffer (pH 6.0), capillary 520 mm x 0.050 mm I.D., and applied voltage - 20 kV. The effective capillary length was studied under the conditions specified above except that the I.D. of capillaries was 0.075 mm and the separation temperature 30 °C. The influence of the applied voltage was studied in 0.03 M phosphate buffer (pH 7.6) inside an 820 mm x 0.075 mm I.D. capillary. The temperature was 30 °C. In each buffer 10 mM CTAB provided the micelle former.

Procedure

A new capillary was successively purged with 0.1 M NaOH, water, and buffer solution for 3x30 minutes. Before each injection the capillary was purged for 2 minutes with the buffer solution.

TABLE 1
Effect of Temperature, Effective Capillary Length, and Applied Voltage on the Current Inside the Capillary.

A Temperature °C	Current μA	B Length cm	Current μA	C Voltage kV	Current μA
25	80	52	200	21	68
30	90	62	170	23	74
35	100	72	140 and 145	25	81
40	110	82	125	27	89
45	120	92	110	29	106

The conditions of analyses were: A) 0.125 M acetate buffer with 10 mM CTAB (pH 6.0), capillary 520 mm x 0.050 mm I.D., and applied voltage -20 kV; B) 0.125 M acetate buffer with 10 mM CTAB (pH 6.0), I.D. of capillaries 0.075 mm, applied voltage -20 kV, and temperature 30 °C; C) 0.03 M phosphate buffer with 10 mM CTAB (pH 7.6), capillary 820 mm x 0.075 mm I.D., and temperature 30 °C.

RESULTS AND DISCUSSION

The effect of the separation temperature on t'_r-values of nine β-blockers and on the t_0-value for each run was studied at 25, 30, 35, and 40 °C. As the temperature was raised the current inside the capillary increased linearly (Table I), probably due to the viscosity and diffusion changes of the sample molecules and of the separation buffer. At the same time the migration of methanol slowed (Figure 1 A). The corrected migration times of the nine β-blockers decreased as the temperature rose from 25 to 35 °C and then stayed constant (Figure 2). The migration window (difference between the first and last compounds) of the β-blockers remained almost unchanged as the temperature was increased from 25 °C to 40 °C. The decrease in migration

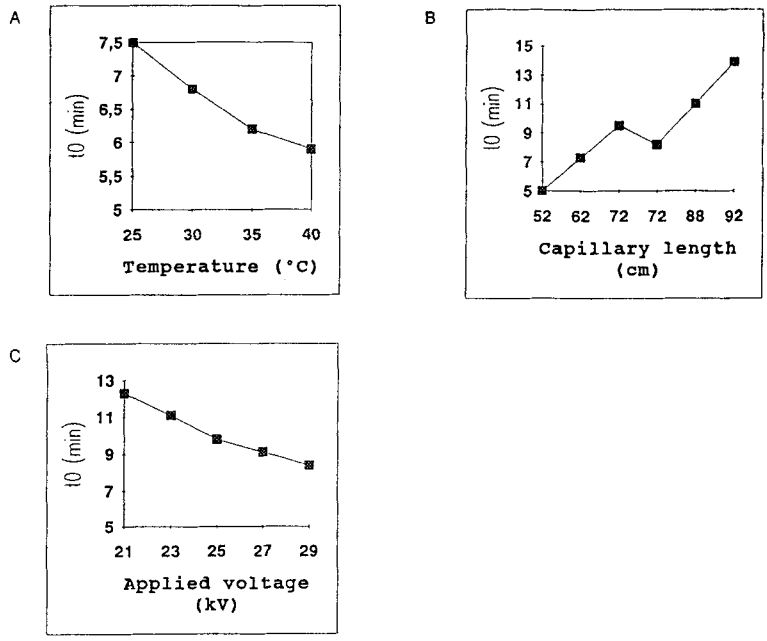

Figure 1.
Effect of A) temperature B) effective capillary length, and C) applied voltage on the electroosmotic breakthrough time (t_0).

times with increasing temperature probably was due to decrease in the viscosity of the buffer solution and the current fluctuation, which in turn were due to joule heating in the capillary. Temperature is therefore a crucial factor, which must be kept constant for reproducible results. Temperatures above 40 °C were studied as well, but the high currents inside the capillary caused baseline disturbances; in addition, the resolution was very low. We were unable to achieve stable temperature conditions below 25 °C.

In another set of experiments, the effective lengths of the capillary tubes were increased from 52 cm up to 92 cm by 10 cm at a time. Ten centimeters

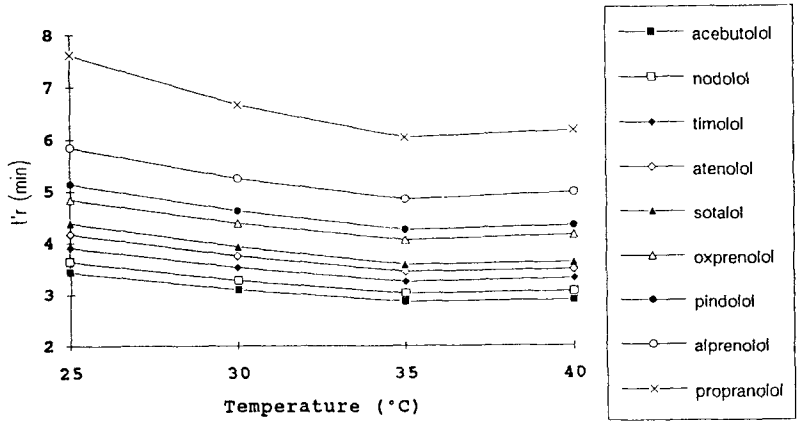

Figure 2.
The corrected migration times of nine β-blockers as a function of temperature.

is the length of one turn on the mandrel inside the capillary cartridge.

Experiments were performed at 30 °C with the constant voltage of - 20 kV. The current decreased with the increase in capillary length (Table I), due to the increasing resistance between the electrodes. The t_o-values increased from 5.0 to 14.0 minutes as the capillary tube was lengthened from 52 cm to 92 cm (Figure 1 B). Likewise, the corrected migration times of the β-blockers increased (Figure 3). As expected, the migration window was twice as wide in the capillary of 92 cm as in that of 52 cm. Although the capillaries used in the study were from the same manufacturer there was considerable difference in their quality, as can be seen in Figure 3. When the first capillary was replaced by another, the current was reduced and the migration times were over 1.5 minutes shorter, even though the separation conditions and the pretreatment of the capillary were unchanged.

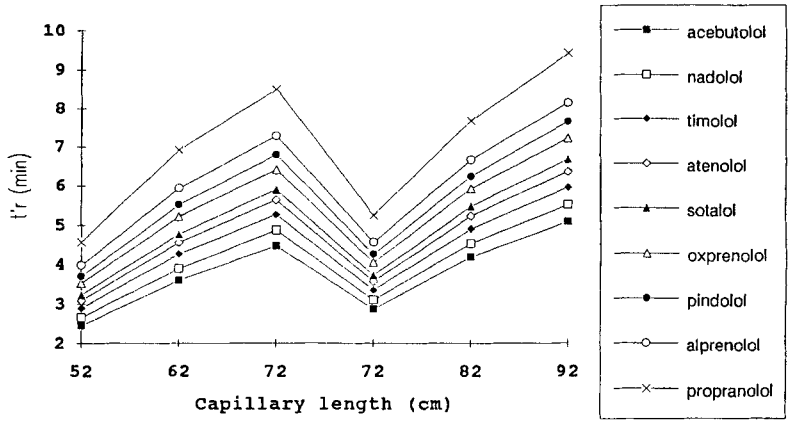

Figure 3.
The corrected migration times of nine β-blockers as a function of effective length of capillary.

The influence of applied negative voltage on the migration times of β-blockers and on the electroosmotic breakthrough time was studied at voltages from - 21 to - 29 kV, with a capillary length of 82 cm and constant separation temperature of 30 °C. As was readily apparent, the current inside the capillary increased with the voltage (Table I), resulting in smaller the t_o-values (Figure 1 C). The corrected migration times of the β-blockers fell off with the increase in voltage from - 21 to - 27 kV, and then began to increase slightly (Figure 4). The slower migration times were due to the stronger electroosmotic flow. Increase in voltage occasioned only a small change in the migration window of the nine β-blockers.

In conclusion, reliable analysis of β-blockers by MEKC requires that temperature, applied voltage, and length of the capillary be carefully

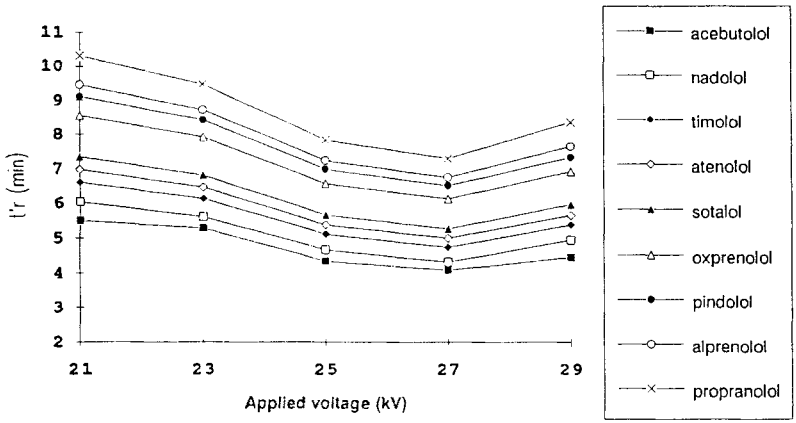

Figure 4.
The corrected migration times of nine β-blockers as a function of the applied voltage.

optimized. MEKC is then a highly attractive method, with either acetate or phosphate as buffer. Temperature control would seem to be of critical importance in quantitative analysis of β-blockers in biological fluids and we intend to utilize the results of this study in the development of a method for the determination of β-blockers in human serum.

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