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REGULATION OF SELECTIVITY IN THE SEPARATION OF PAFENOLOL AND POTENTIAL IMPURITIES BY REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

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SUMMARY

A liquid chromatographic system for the isolation of impurities in a beta-adrenoreceptor antagonist, pafenolol, is described. The retention of the solutes is regulated by 1-pentanol, N,N-dimethyloctylamine and 3,5-dimethylcyclohexyl sulphate in a mobile aqueous buffer solution. LiChrosorb RP-8 is used as stationary phase.

The selectivity of the separation systems is influenced by the concentration of the mobile-phase components, reflecting the different properties of the solutes. The correct composition of the mobile phase gives complete resolution of the sample components.

The proposed liquid chromatographic method enables determination by UV monitoring of less than 0.1% of each of the impurities in pafenolol.

INTRODUCTION

Reversed-phase ion-pair chromatography is an universally applied technique for the separation of amines. However, hydrophobic amines give badly tailing peaks in many systems unless precautions are taken[1–4]. The effect has been attributed to residual silanol groups on the surface of the hydrophobized solid phase, and efforts have been made to overcome the problem, for example by endcapping[5,6]. This has sometimes had an adverse effect, and unsatisfactory chromatographic performance has been observed even for hydrophilic amines[7].

The most successful approach to improving the peak shape and column efficiency for solutes containing amino groups has been to include an organic amine as a modifier in the acidic aqueous eluent[1–4]. The amine strongly decreases the retention of cationic solutes by competing for the binding capacity of the adsorbent. The competing effect is highly dependent on the structure of the amine as regards both retention and chromatographic performance[2].

The characterization of the potential impurities and degradation products of drug substances is an important part of pharmaceutical analysis. The separation of these related compounds, often present in very low concentrations, requires highly

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selective and efficient chromatographic systems. Thin-layer chromatography has been used for this purpose with beta-adrenoreceptor antagonists[8,9], and recently reversed-phase liquid chromatography has been applied both in pharmaceutical analysis[10,11] and in bioanalysis[12,13].

This paper describes ion-pair liquid chromatographic systems for the isolation and quantitation of potential impurities in pafenolol. Attention has been paid to regulation of retention and selectivity by varying the content of the modifiers and the counter-ion in the mobile phase. The systems used are highly flexible and can easily be adapted for other beta-adrenoreceptor antagonists.

EXPERIMENTAL

Chemicals and reagents

N,N-Dimethyloctylamine (DMOA) was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and distilled before use. N,N,N′,N′-Tetramethylethylenediamine (TMEA) was obtained from E. Merck (Darmstadt, G.F.R.) and 1-pentanol from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Potassium 3,5-dimethylcyclohexyl sulphate (DMCHS) and pafenolol and other substances being studied (Fig. 1), were supplied by the Department of Organic Chemistry, AB Hässle. All other chemicals and reagents were of analytical grade and were used without further purification.

Apparatus

Photometric measurements were performed on a Beckman Acta CIII recording spectrophotometer with 10-mm quartz cells. The pH measurements were performed on a Radiometer PHM 64 research pH meter with a Radiometer combined electrode. An Altex Solvent Metering Pump, Model 110A, was used to generate a flow-rate of 1.0 ml/min. Samples were applied to the chromatographic column by a Rheodyne injector with 20- or 100-μl loops. The Pye Unicam LC 3 and the LDC

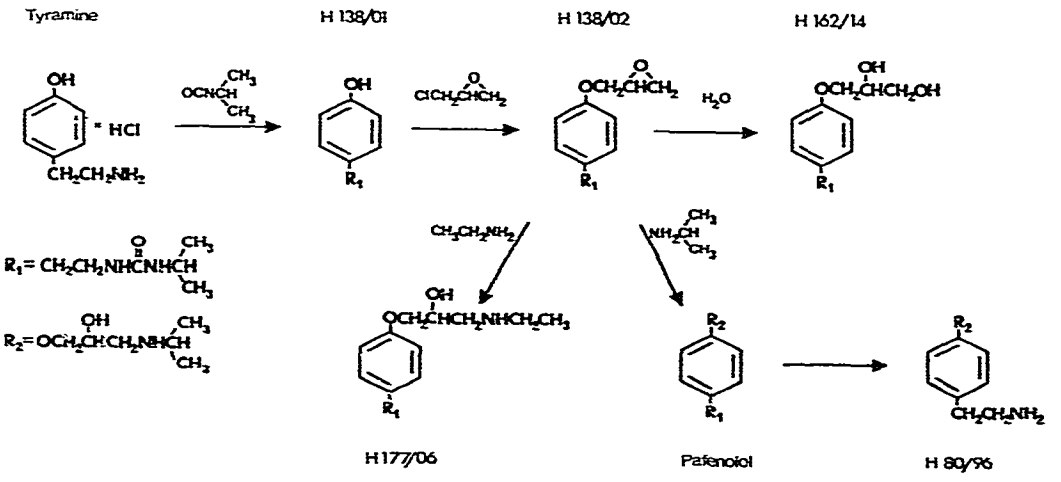

Fig. 1. Synthesis of pafenolol.

SpectroMonitor III UV detectors were operated at 270 nm, at which wavelength all of the compounds studied exhibited molar absorptivities in the range $1 \cdot 10^3$–$2 \cdot 10^3$. The columns were made of stainless steel (150 × 4.0 mm I.D.) and were equipped with modified Swagelok connectors and packed with LiChrosorb RP-8, 5 μm (E. Merck). Ambient temperature (23 ± 1°C) was used throughout the experiments.

Chromatographic system

The mobile phases were prepared by dissolving appropriate amounts of the counter-ion, DMCHS, and the modifiers, 1-pentanol and TMEA or DMOA, in 0.1 *M* phosphoric acid. The pH was adjusted to 2.1–2.2 by addition of sodium hydroxide. The column was equilibrated with mobile phase until a stable baseline was obtained, and repeated injections of samples gave constant retention times. Mobile phase diluted with water (1:1) was used to estimate the volume of eluent in the column, V_m.

Before a new mobile phase of a different composition was introduced, the column was washed with 100 ml of methanol. This enabled rapid and reproducible conditioning even for mobile phases containing 1-pentanol. Methanol is assumed to facilitate wetting of the hydrophobic stationary phase with the aqueous phase.

Quantitative determination of impurities in pafenolol

A 100-μl volume of sample, corresponding to 50 μg of pafenolol, dissolved in mobile phase, was injected into the column. The absorbance of the eluate was measured with a full-scale absorbance of 0.005. Quantitation of separated impurities was made by peak height measurements using a standard curve obtained by injection of reference solutions containing 0.15–2.5 μg/ml of each separated compound.

RESULTS AND DISCUSSION

Synthesis

Pafenolol is a secondary amine with a pK_a of *ca.* 9.5. It is synthesized from tyramine in three steps[14], as illustrated in Fig. 1. The intermediate phenol, H 138/01, is a potential impurity in pafenolol and so are the intermediate epoxide, H 138/02, and the diol, H 162/14, produced by the reaction between H 138/02 and water. Aminolysis of the urea group in the molecule to give the degradation product H 80/96, might take place in the third step, which also may introduce by-products such as H 177/06 arising from impurities in the isopropylamine. These compounds, with the exception of H 138/02 (capacity ratio, $k' > 20$), were included as solutes in the development of the chromatographic method.

The chromatographic system

The chromatographic principle applied in this study has been thoroughly investigated in recent papers[2,3,15]. The mobile phase is a phosphate buffer (pH 2.2) with addition of organic modifiers and counter-ions. Adequate retention of the most hydrophilic amines, tyramine and H 80/96, was achieved by use of DMCHS as counter-ion. Four of the compounds studied are amines and distributed as ion pairs to the adsorbing phase. The other two are uncharged and are retained as such at pH 2.2. The neutral solutes and the amines, pafenolol and H 177/06, are strongly retained with 10^{-2} *M* DMCHS in phosphate buffer as the mobile phase, whereas the amines H

TABLE I

EQUATIONS FOR REGULATION OF CAPACITY RATIOS

Q = modifier; HB = amine solute; S = neutral solute; Pe = 1-pentanol.

Solute	*Cationic modifier*	*Capacity ratio*	*Eqn. number*
HB^+	Q^+	$k'_{HB} = \frac{qK_0K_{HBX}[X^-]_m}{1 + K_{QX}[Q^+]_m[X^-]_m + K_{Pe}[Pe]^n_m}$	(1)
HB^+	Q^{2+}	$k'_{HB} = \frac{qK_0K_{HBX}[X^-]_m}{1 + K_{QX_2}[Q^{2+}]_m[X^-]^2_m + K_{Pe}[Pe]^n_m}$	(2)
HB^{2+}	Q^+	$k'_{H_2B} = \frac{qK_0K_{H_2BX}[X^-]^2_m}{1 + K_{QX}[Q^+]_m[X^-]_m + K_{Pe}[Pe]^n_m}$	(3)
HB^{2+}	Q^{2+}	$k'_{H_2B} = \frac{qK_0K_{H_2BX}[X^-]^2_m}{1 + K_{QX_2}[Q^{2+}]_m[X^-]^2_m + K_{Pe}[Pe]^n_m}$	(4)
S	Q^+	$k'_S = \frac{qK_0K_S}{1 + K_{QX}[Q^+]_m[X^-]_m + K_{Pe}[Pe]^n_m}$	(5)
S	Q^{2+}	$k'_S = \frac{qK_0K_S}{1 + K_{QX_2}[Q^{2+}]_m[X^-]^2_m + K_{Pe}[Pe]^n_m}$	(6)

80/96 and tyramine are only moderately retained. The addition of a neutral modifier, 1-pentanol, or a cationic modifier, DMOA or TMEA, decreased the retention of the hydrophobic solutes to suitable retention times.

Retention principles

Recent studies[2,3,15–17] have shown that the retention of amines on chemically bonded phases follows a model based on adsorption as ion pairs on two different sites on the solid phase. The capacity ratio of an univalent amine, HB^+, on one of the sites, can be calculated from eqn. 1 (Table I), where q is the ratio of the weight of solid phase to the volume of eluent in the column and K_0 is the total capacity of the adsorbing phase. The amine, HB^+, is assumed to be adsorbed as an ion pair with a counter-ion, X^-, in an equilibrium between mobile phase and solid phase given by the equilibrium constant, K_{HBX}. The term $K_{QX}[Q^+]_m[X^-]_m$ expresses the influence of a cationic modifier, Q^+_m, and the term $K_{Pe}[Pe]^n_m$ allows for the influence of 1-pentanol on the capacity ratio of HB^+. Eqn. 1 is valid under the assumption that adsorbed HBX makes a negligible contribution to K_0. From eqn. 1 it follows that the capacity ratio is

dependent on the concentration of the mobile phase components Q_m^-, X_m^- and Pe_m. Depending on the properties and concentrations of these components, the counter-ion, the cationic modifier or the neutral modifier will have a predominant influence on the capacity ratio of the sample amine.

Equations for systems containing charged and uncharged solutes and modifiers can be derived according to principles given in ref. 3 and used for predictions of the influence of the mobile-phase components on the capacity ratio. Eqns. 1–6 in Table I cover the different separation systems used in the present investigation.

Influence of 1-pentanol on retention

Studies on LiChrosorb RP-8 and LiChrosorb RP-18 have shown that 1-pentanol at concentrations below 2% in an aqueous mobile phase is adsorbed to an extent that corresponds to a monolayer on the surface of the adsorbing phase[17,18]. According to a recent study, methanol and acetonitrile, which are more commonly used as modifiers, are assumed to be adsorbed in a mixture of constant composition[19] with water. In our study, 1% of 1-pentanol gave capacity ratios of the same magnitude as did 20% of acetonitrile (Fig. 2). No significant change in selectivity was obtained by use of acetonitrile instead of 1-pentanol as modifier in the ion-pair system studied.

The effect of increasing the mobile-phase concentration of 1-pentanol is illustrated in Fig. 3. The logarithmic plot indicates a stronger effect on the amines pafenolol, H 177/06 and H 80/96 than on the uncharged H 138/01 and H 162/14. A mobile phase containing 0.025 *M* TMEA was used. Corresponding capacity ratios were obtained by use of DMOA in a concentration of $5 \cdot 10^{-4}$ *M*. With a higher concentration of DMOA ($3 \cdot 10^{-3}$ *M*) the competing effect of 1-pentanol was of the same magnitude on all solutes studied. This is probably due to the fact that adsorption sites with a high affinity for amines are already covered with strongly adsorbed DMOA[3,15].

Cationic modifiers

The influence of the univalent cationic modifier DMOA on the retention of the compounds studied is illustrated in Fig. 4. Increasing the concentration of DMOA results in decreased capacity ratios of neutral, univalent and bivalent solutes, in accordance with eqns. 5, 1 and 3, respectively.

DMOA has a stronger effect on the amines than on the neutral solutes H 138/01 and H 162/14, probably owing to a selective competition for the adsorption sites with a high affinity for amines. A difference in the effect of DMOA on the amines is also demonstrated in Fig. 4. The influence of DMOA is stronger on the bivalent amine H 80/96 than on the univalent amines pafenolol, H 177/06 and tyramine.

Counter-ion effects

Selective effects on the capacity ratios can be obtained even by changing the concentration of the counter-ion in the eluent. The influence of DMCHS on the retention of the compounds studied is shown in Fig. 5. The retention of the neutral solutes decreases slightly while the retention of the amines increases with increasing concentration of the counter-ions. The decrease in the capacity ratios of the neutral solutes is probably due to displacement by an increased amount of adsorbed DMOA, as predicted by eqn. 5. The weak increase in the retention of the univalent amines as ion pairs is also due to a competing adsorption of DMOA (*cf.* eqn. 1).

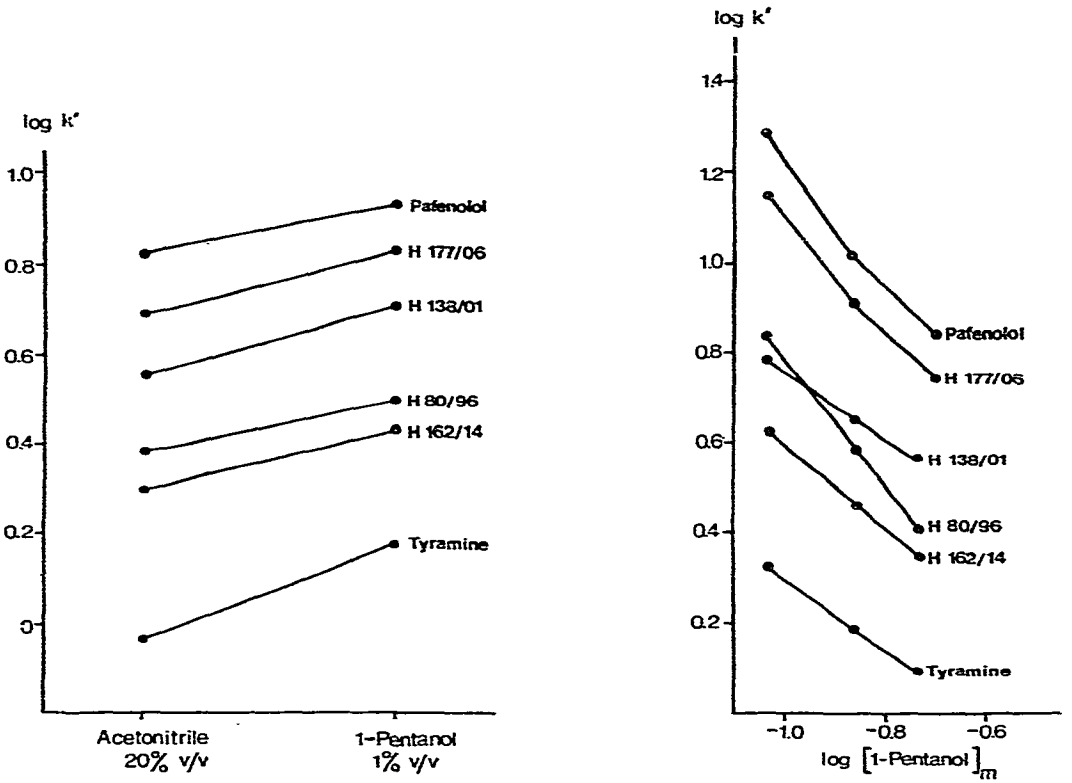

Fig. 2. Effect of the nature of the organic solvent on the capacity ratios. Mobile phase: $1 \cdot 10^{-2}$ *M* DMCHS and $1 \cdot 10^{-3}$ *M* DMOA in phosphate buffer (pH 2.2) with addition of acetonitrile (20%) or 1-pentanol (1%). Support: LiChrosorb RP-8.

Fig. 3. Regulation of the retention by varying the concentration of 1-pentanol in the mobile phase. Mobile phase: 1-pentanol. $1 \cdot 10^{-2}$ *M* DMCHS and $2.5 \cdot 10^{-2}$ *M* TMEA in phosphate buffer (pH 2.2). Support: LiChrosorb RP-8.

Quantitative determination

Suitable separation conditions can be obtained from plots of capacity ratios against the concentration of organic modifiers (Figs. 3 and 4). Separation of pafenolol and the impurities studied can, according to Fig. 4, be performed when the concentration of DMOA is *ca.* 10^{-3} *M*, as demonstrated by the chromatogram in Fig. 6. This chromatographic system with $1.5 \cdot 10^{-2}$ *M* DMCHS and $9.2 \cdot 10^{-2}$ *M* 1-pentanol (*i.e.* 1% of 1-pentanol) in phosphate buffer was selected as the mobile phase in the quantitative determination of impurities in pafenolol. Owing to interference from small amounts of unidentified impurities in the sample, and also to lot-to-lot variations in the properties of the support, it is often necessary to make a final adjustment to the separation conditions. The best result is usually obtained by a small change in the amount of DMCHS added, as follows from Fig. 7, which illustrates the effect of DMCHS in the concentration range $1.0 \cdot 10^{-2}$–$1.5 \cdot 10^{-2}$ *M* on the capacity ratios. An unidentified impurity interfering with the separation of H 162/14 and H

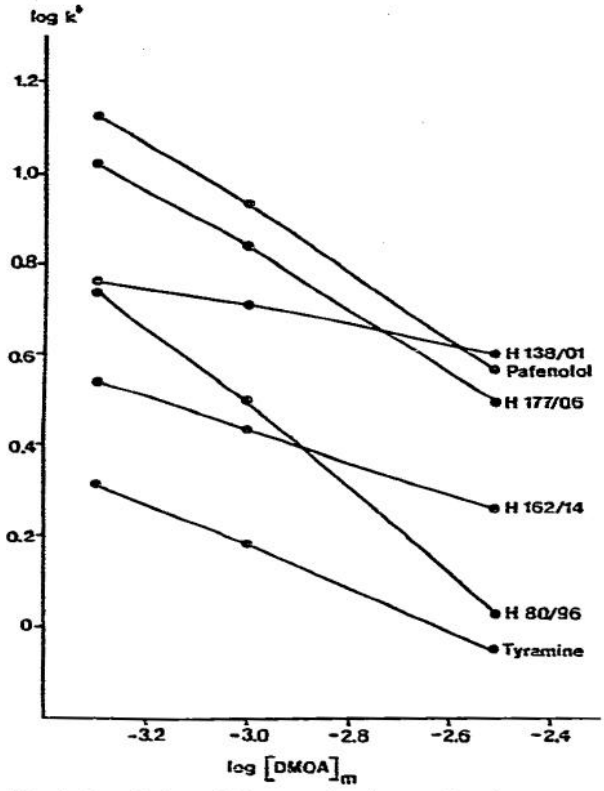

Fig. 4. Regulation of the retention by varying the concentration of DMOA in the mobile phase. Mobile phase: DMOA, $1 \cdot 10^{-2}$ *M* DMCHS and $9.2 \cdot 10^{-2}$ *M* 1-pentanol in phosphate buffer (pH 2.2). Support: LiChrosorb RP-8.

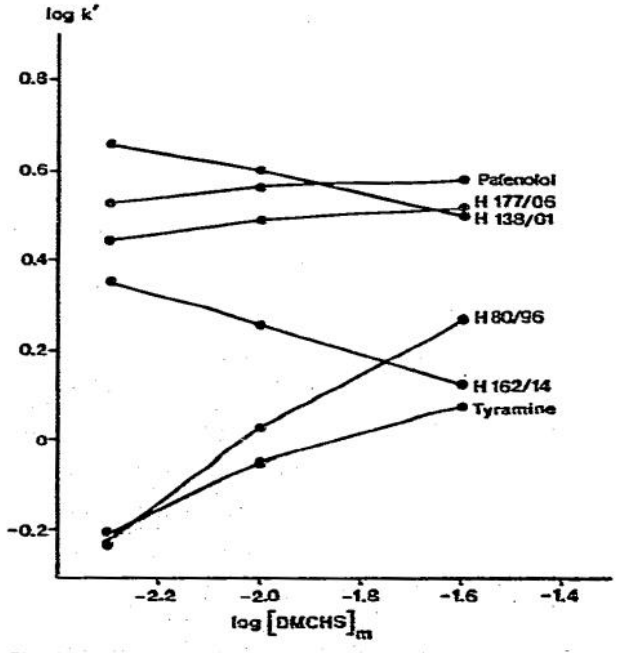

Fig. 5. Regulation of the retention by varying the concentration of DMCHS in the mobile phase. Mobile phase: DMCHS, $3 \cdot 10^{-3}$ *M* DMOA and $9.2 \cdot 10^{-2}$ *M* 1-pentanol in phosphate buffer (pH 2.2). Support: LiChrosorb RP-8.

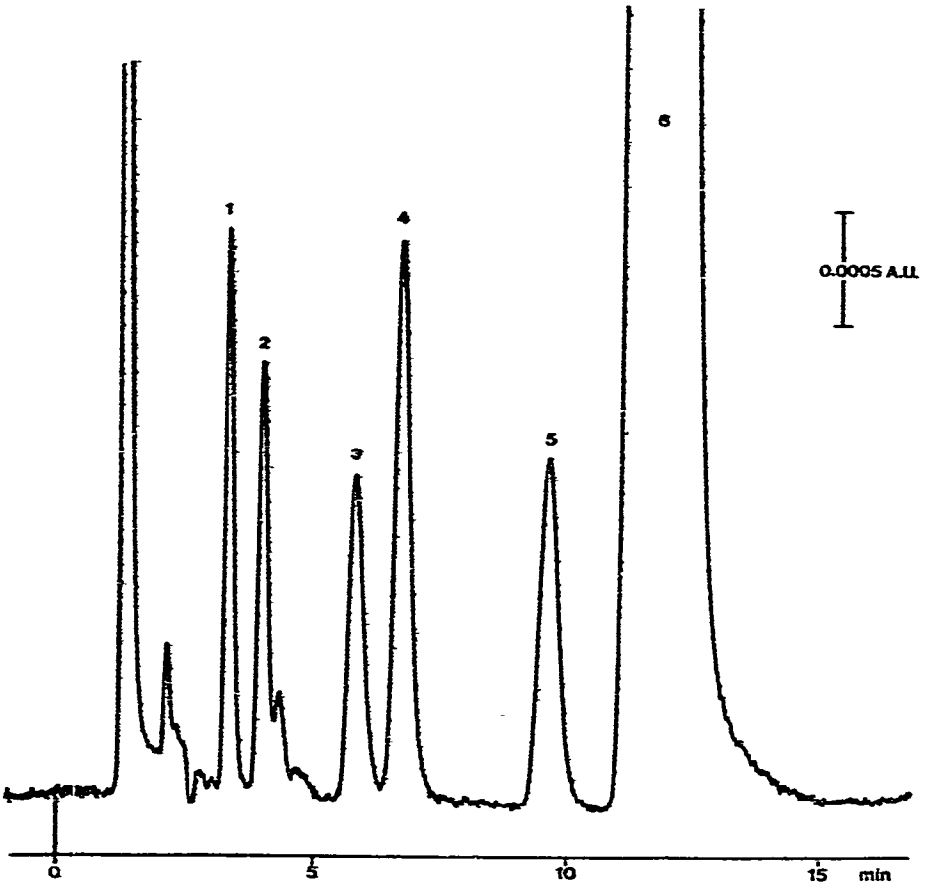

Fig. 6. Separation from pafenolol of *ca.* 0.3% of each of the impurities studied. Mobile phase: $1 \cdot 10^{-3}$ *M* DMOA, $1.5 \cdot 10^{-2}$ *M* DMCHS and $9.2 \cdot 10^{-2}$ *M* 1-pentanol in phosphate buffer (pH 2.2). Support: LiChrosorb RP-8. Flow-rate: 1.0 ml·min. Detection wavelength: 270 nm. Samples: 1 = tyramine; 2 = H 162.14; 3 = H 80,96; 4 = H 138,01; 5 = H 177,06; 6 = pafenolol.

TABLE II

DETERMINATION OF REPRODUCIBILITY BY INJECTION OF TWO SAMPLE SOLUTIONS OF PAFENOLOL

Substance	*Sample 1*			*Sample 2*		
	Amount (μg)	*Percentage of pafenolol*	*Coefficient of variation (%), n = 10*	*Amount (μg)*	*Percentage of pafenolol*	*Coefficient of variation (%), n = 10*
Tyramine	0.042	0.08	1.39	0.014	0.03	2.21
H 162,14	0.070	0.14	1.95	0.023	0.05	3.42
H 80,96	0.098	0.20	1.78	0.033	0.07	2.11
H 138,01	0.082	0.16	1.86	0.027	0.05	2.90
H 177,06	0.15	0.3	1.50	0.045	0.09	3.06

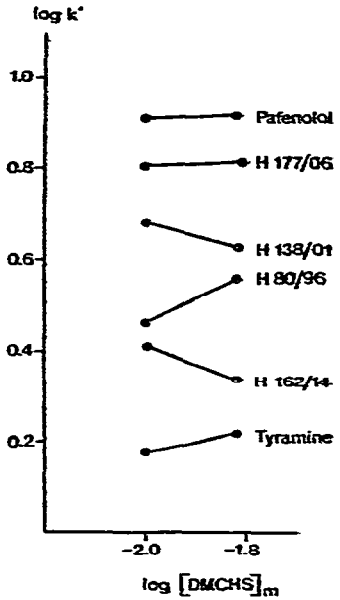

Fig. 7. Regulation of the retention by varying the concentration of DMCHS in the mobile phase. Mobile phase: DMCHS, $1 \cdot 10^{-3}$ *M* DMOA and $9.2 \cdot 10^{-2}$ *M* 1-pentanol in phosphate buffer (pH 2.2). Support: LiChrosorb RP-8.

80/96 with $1.0 \cdot 10^{-2}$ *M* DMCHS in the mobile phase was separated, close to H 162/14, with $1.5 \cdot 10^{-2}$ *M* DMCHS, as shown in the chromatogram (Fig. 6).

The standard curves used in the quantitation showed linearity in the concentration range studied (0.15–2.5 μg/ml). The minimum detectable amounts were below 0.1 μg/ml for all impurities studied, 100 μl being injected.

The reproducibility of the method was determined by ten injections of each of two sample solutions containing 0.05–0.1 % and 0.1–0.3 % of each impurity added to pafenolol. The coefficients of variation were in the ranges 2.1–3.4 % and 1.5–2.0 %, respectively (Table II).

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