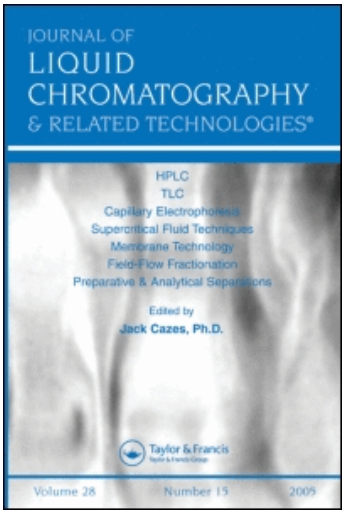

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Effect of Temperature on HPLC Separations of Penicillins

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EFFECT OF TEMPERATURE ON HPLC SEPARATIONS OF PENICILLINS

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ABSTRACT

Reversed-phase high performance liquid chromatography (RP-HPLC) was used to separate in the same sample the following penicillins : amoxicillin, ampicillin, piperacillin, penicillin G, penicillin V and cloxacillin. After the chromatographic separation in isocratic elution conditions of the penicillins at ambient temperature, the effect of the column temperature on resolution was analyzed. The capacity factor of each penicillin was observed to increase with decreasing temperature, a linear relationship was obtained for a plot of ln k' versus 1/T. The results also showed changes in resolution between adjacent peaks being associated with differences in the selectivity factor (α).

INTRODUCTION

The effect of temperature as a separation parameter in RP-HPLC is often neglected. The reasons for this is that the range of temperature available is practically limited to 5 - 100°C and that

for many systems the solvent eluotropic strength is not sensitive to temperature. On the other hand,very important changes in the capacity factors of solutes can be realized by altering the composition of the mobile phase. However, in some systems the temperature is a very important parameter (1-9).

RP-HPLC has been shown to be well suited for the analysis and separation of penicillins (10-12); in all cases the separation is carried out at ambient temperature and, to date, a search of the literature reveals that there has not been a study comparing the effect of temperature on HPLC separations of penicillins.

This paper describes the effect of column temperature on the resolution in isocratic mode of some penicillins with reversed phase HPLC. This study was motivated because we observed a difference in resolution with day-to-day changes in ambient temperature.

MATERIALS AND METHODS

Materials

The β-lactam antibiotics were supplied by Antibióticos S.A. (Madrid, Spain), except piperacillin that was supplied by Lederle Laboratories (Pearl River, NY 10965, U.S.A.). Their structures are shown in table 1.

HPLC-grade water and isopropanol (Carlo Erba, Milán, Italy) were used in this study. All other chemicals (analytical grade) were obtained from Merck (Darmstadt, F.R.G.)

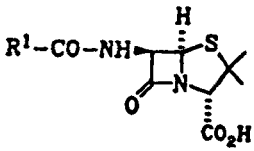
Instruments

The HPLC system consisted of a Konik KNK-500-A liquid chromatograph, a Rheodyne Model 7125 loop injector (volume 20 µl), a Waters Model 441 UV detector and a Varian Model 4290 computing integrator. The chromatograph was equipped with a Spherisorb ODS column (10 µm particle size; 25 cm x 4.9 mm I.D.).

Temperature control : A constant column temperature was achieved by putting the column into a temperature-controlled water-bath. The temperature of the separation column was controlled at ± 0.2°C.

TABLE 1

Structures of Penicillins



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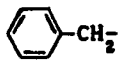
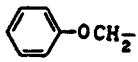
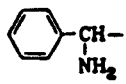
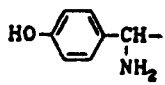
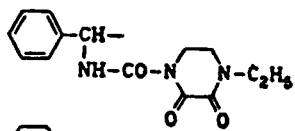
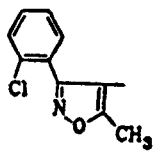
	Penicillin G
	Penicillin V
	Ampicillin
	Amoxicillin
	Piperacillin
	Cloxacillin

Chromatographic procedure

The mobile phase used to separate the compounds was a mixture of aqueous 25 mM KH_2PO_4 + 25 mM K_2HPO_4 solution/isopropanol : 80/20 (v/v) (final pH 7.25).

The β-lactam antibiotics were dissolved in mobile phase. The flow-rate of the mobile phase was 1.0 ml/min and the capacity factors were calculated according to the equation :

$$k' = \frac{(t_r - t_o)}{t_o}$$

where t_r and t_o are the average retention time of the solute and of a non-retained substance respectively. The column dead time, t_o, was measured by injecting methanol.

A pre-column (3 cm x 4.6 mm I.D.) packed with the same packing materials was used to guard the main column. The detector was set at 229 nm.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms for the mixture of penicillins obtained when the mixture was chromatographed at each of the five column temperatures from 15 to 55 °C. As one can see a marked temperature effect is produced on the chromatographic behavior of each penicillin. As the temperature increases the retention time of each of the peaks decreases, while at the lower temperatures the retention time increases, resulting in better resolution of each of the peaks at 25 - 35 °C. The rate of change of retention time was not the same for each penicillin. This change was highest for the penicillin V and cloxacillin.

In table 2 are shown the retention times and capacity factors (k') of each penicillin at each temperature. The values are averages from five replicate determinations. The variation coefficients were generally less than 2 %. The dependence of the capacity factor on the temperature is given by the well-known van't Hoff equation :

$$\ln (k') = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \Phi$$

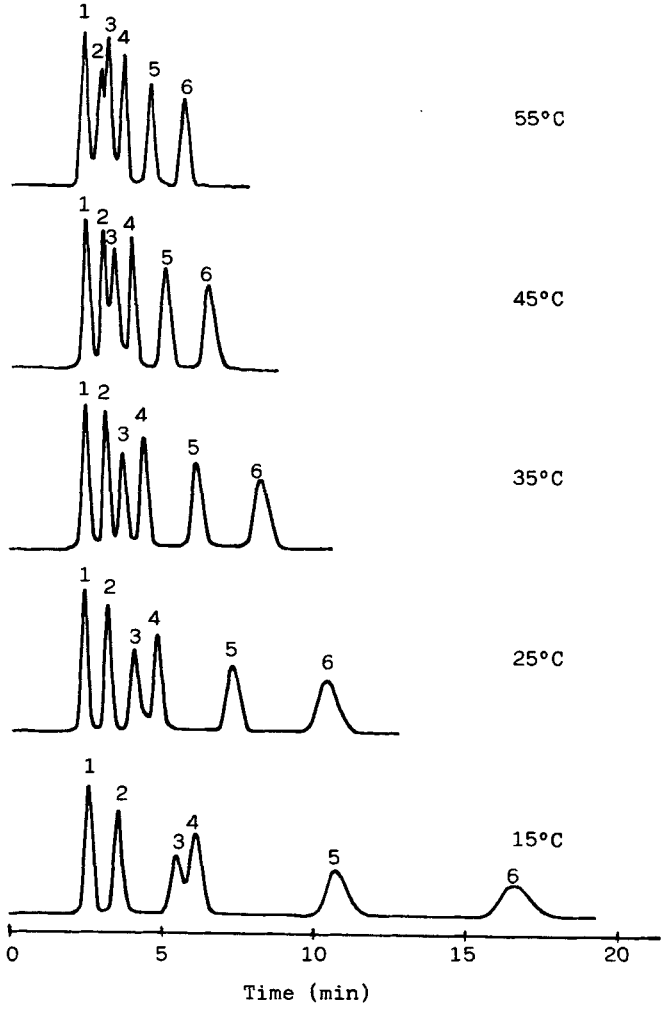


FIGURE 1. Effect of column temperature on the elution profile of 1 = amoxicillin, 2 = ampicillin, 3 = piperacillin, 4 = penicillin G, 5 = penicillin V and 6 = cloxacillin using a mobile phase of 50 mM phosphate buffer/isopropanol: 80/20 (v/v) (final pH 7.25) at flow-rate of 1 ml/min.

TABLE 2

Effect of Column Temperature on the Capacity Factor (k') of the Penicillins.

Penicillin	T (°C)	Average Ret. Time (min)	k'	Variation Coefficient (%)
Amoxicillin	15	2.56	0.113	1.3
	25	2.47	0.097	1.1
	35	2.40	0.081	4.2
	45	2.36	0.072	0.5
	55	2.31	0.065	2.7
Ampicillin	15	3.51	0.526	1.4
	25	3.24	0.440	2.5
	35	3.07	0.382	0.6
	45	2.94	0.336	1.2
	55	2.84	0.309	1.7
Piperacillin	15	5.49	1.387	0.7
	25	4.18	0.857	1.5
	35	3.66	0.648	2.1
	45	3.29	0.495	1.0
	55	3.01	0.387	3.8
Penicillin G	15	6.12	1.660	2.1
	25	5.01	1.226	0.2
	35	4.37	0.968	0.8
	45	3.89	0.768	1.3
	55	3.58	0.650	2.1
Penicillin V	15	10.75	3.674	2.7
	25	7.58	2.368	2.1
	35	6.14	1.766	0.7
	45	5.05	1.295	0.3
	55	4.36	1.009	0.6
Cloxacillin	15	16.49	6.169	1.1
	25	10.78	3.791	4.6
	35	8.25	2.716	2.0
	45	6.47	1.941	0.7
	55	5.41	1.493	1.4

where R is the gas constant, ΔH° and ΔS° are the enthalpy and entropy changes, respectively, associated with the solute retention process. The parameter Φ is the phase ratio and T is the absolute temperature. As the temperature increases, the values of the capacity factor, k', of the penicillins studied decrease as shown in table 2.

The van't Hoff plots for all penicillins studied gave straight lines (figure 2), each with a correlation coefficient of 0.997 . The apparent linearity of the plots supports the assumption that single sorption mechanisms are operative for each penicillin. These lines, however, were not all parallel as one might expect if the effect of temperature was merely a generalized one. As one can see there are three different slopes that can be ascribed to probably different separation mechanisms. Because of their relative positions in the chromatogram, there is a change in the resolution of piperacillin from ampicillin and from penicillin G over the temperature range between 15 - 55 °C.

The values of enthalpy changes can be determined since $-\Delta H^\circ/R$ are the slopes of the plots. In all cases studied (table 3) the values of enthalpy are negative, this indicates that the transfer of solute from the mobile phase to sorption sites is favoured.

TABLE 3

Enthalpy Changes for the Penicillins Studied.

Penicillin	ΔH° (KJ/mole)
Amoxicillin	-11.15
Ampicillin	-10.52
Piperacillin	-24.43
Penicillin G	-18.43
Penicillin V	-25.08
Cloxacillin	-27.60

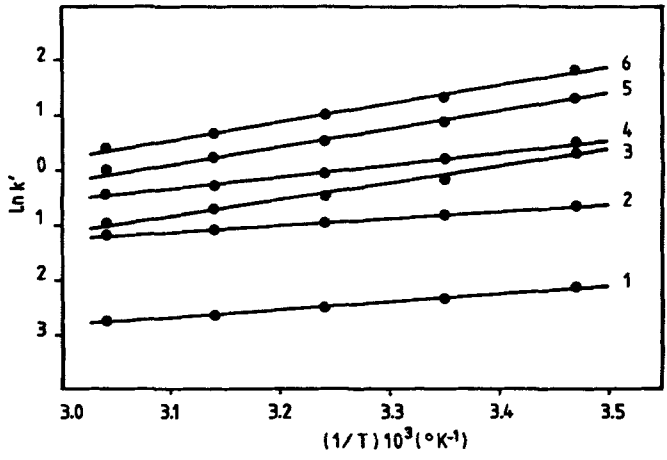


FIGURE 2. Effect of column temperature on the capacity factor, k', of penicillins. Mobile phase, flow-rate and numbering as in fig. 1.

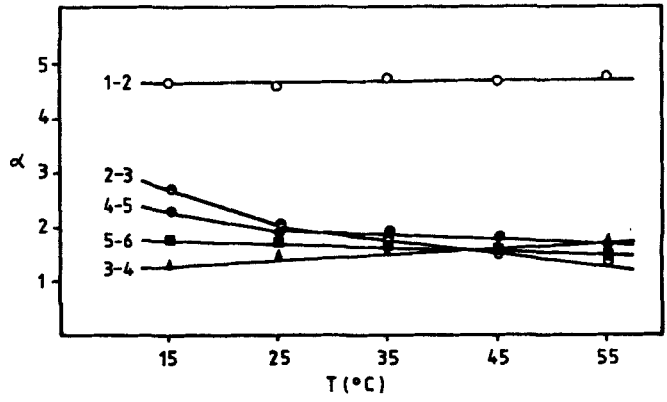


FIGURE 3. Effect of column temperature on selectivity factor, α, of the five pairs of sequentially resolved peaks. Mobile phase, flow-rate and numbering as in fig. 1.

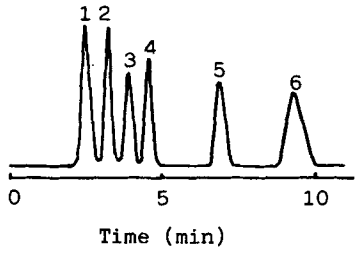


FIGURE 4. Elution profile under the optimal conditions. Column temperature 30°C. Mobile phase, flow-rate and numbering as in fig. 1.

The influence of the temperature changes on the selectivity factors, α, are shown in figure 3. In the 2 - 3 pair the selectivity factor decreases with an increase in temperature. In the 3 - 4 pair the temperature increase is beneficial throughout the whole temperature range, as shown by the slight increase in α. In the 1 - 2, 4 - 5 and 5 - 6 pairs the selectivity factor is affected little by temperature changes.

During the entire study, we observed no deterioration of the column used Spherisorb ODS. At present, the life time of the column under the analysis conditions used has not been determined.

CONCLUSION

The effect of temperature changes on separation of penicillins may be compared to changes in the compositions of mobile phases. Thus, temperature changes may substitute in some cases for changes in mobile phase composition or may be complementary to them.

Temperatures of about 30ºC improved resolution of this mixture of penicillins. The temperature parameter may be applied to the chromatographic separation of different penicillin mixtures.

Figure 4 shows the separation at 30ºC of a mixture of penicillins using a mobile phase of 50 mM phosphate buffer/isopropanol : 80/20 (v/v) (final pH 7.25). The six penicillins could all be separated and analyzed in less than 10 min.

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