INFLUENCE OF TEMPERATURE AND MOBILE PHASE COMPOSITION ON RETENTION PROPERTIES OF THE MACROLIDE ANTIBIOTICS CLARITHROMYCIN AND ROXITHROMYCIN IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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Abstract

The reversed-phase (RP) chromatographic behaviour of the macrolide antibiotics clarithromycin (Clari) and roxithromycin (Roxi) was extensively studied as a function of mobile phase composition - modified with one, two or three of organic solvents - and column temperature. The results of our study provide a satisfactory thermodynamic explanation for the retention processes of these macrolides in RP-HPLC and give additional information on the selection of optimal separation conditions for the determination of Clari in human plasma using Roxi as an internal standard and vice versa.

Key words: *clarithromycin, roxithromycin, HPLC retention.*

1. Introduction

Clari and Roxi are relatively new semisynthetic macrolide antibiotics, which exhibit better oral bioavailability and a more favorable pharmacokinetic behavior than other macrolides [1]. So far, a limited number of papers can be found in literature concerning the analysis of Clari and Roxi in biomatrices [1-5]. However, no paper has systematically examined the retention of these and other related macrolides under different chromatographic conditions to date. The aim of the present study is to explore extensively the retention behavior of Clari and Roxi under varying mobile phase composition and column temperature, in order to find out the optimal conditions for assay of these drugs.

2.Experimental

The liquid chromatography system used in this investigation is described elsewhere [5]. Two analytical columns were used: a 250 × 4.6 mm Kromasil C_{18} 5 μm and a 250 × 4 mm Inertsil ODS-3 5μm column. The electrochemical detection of the analytes was performed at 1.0 V vs the Ag/AgCl reference electrode. Experiments were performed over the range of temperature from 25 to 70^0 C. The volume flow rate of all mobile phases used was 1.0 ml/min. Binary, ternary and quaternary eluent systems were used. The different mobile phases with constant pH value equal to 7.0 and ionic strength 0.02 M consisted of an aqueous phosphate buffer and one, two or three of the following organic modifiers MeOH, ACN and isopropanol (iPrOH). The compositions of the mobile phases used in this investigation are specified in Fig. 1. Hold-up times for both columns were estimated and found to be 2.26 min for the Kromasil column and 1.81 min for the Inertsil one.

3. Results and discussion

The effect of solvent strength (as controlled by the percentage or the volume fraction, φ, of an organic modifier in a binary mobile phase such as MeOH-aqueous buffer or ACN-aqueous buffer) on the retention of a solute is usually expressed as

$$ln\ k' = A\varphi^2 + B\varphi + C \qquad (1)$$

An extension of Eq.(1) for a ternary mobile phase is

$$ln\ k' = A_1\varphi_1^2 + A_2\varphi_2^2 + B_1\varphi_1 + B_2\varphi_2 + C + D\varphi_1\varphi_2 \qquad (2)$$

The results of the regression analysis of the retention data of Clari and Roxi carried out according to Eq.(1) showed that this equation describes the retention of both macrolides satisfactorily in all eluents used. Note that, Eq.(2) did not provide a better fit to experimental data obtained in a ternary mobile phase system such as ACN-iPrOH-aqueous buffer, since the ratio of the two organic modifiers keeps constant in different concentrations of this ternary eluent system. Moreover, it is obvious from Fig. 1 that plots of *ln k' vs. φ* for Clari are nearly linear when MeOH or MeOH-ACN is used as organic solvents, while ACN and the other binary or ternary combinations of organic solvents give marked curvature. On the other hand, ACN and iPrOH have a higher solvent strength than MeOH as depicted in Fig.1. In conclusion, taking into accound also the results of our previous work [5], among the different mobile phases examined the ternary eluent system consisting of MeOH-ACN-aqueous buffer appears to be favored for assay of Clari and Roxi.

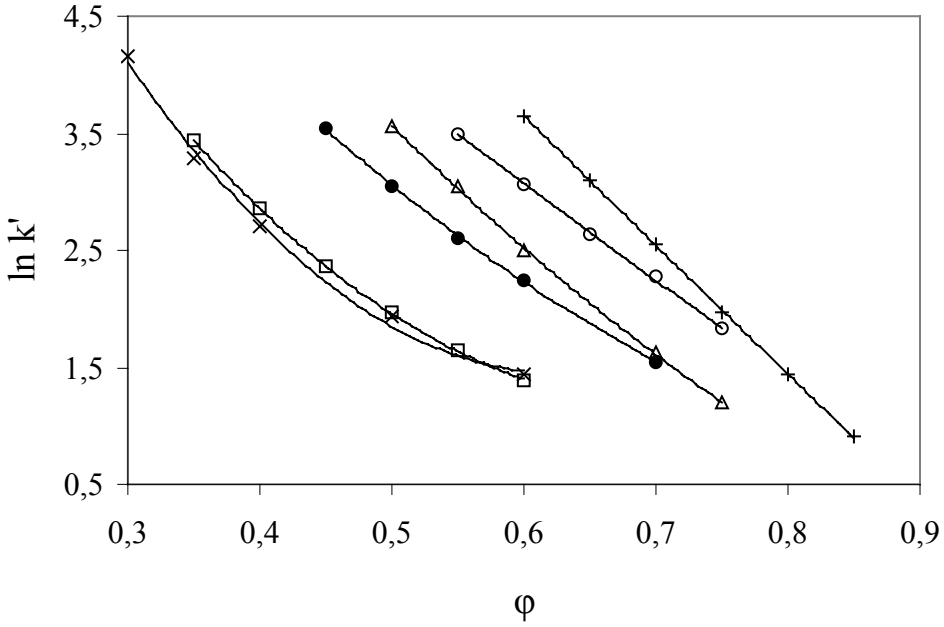

Fig.1. Plots of *ln k' vs. φ* of different organic solvents for Clari eluted from Kromasil column. (+) MeOH; (×) ACN; (o) MeOH-ACN (4:3v/v); (Δ) MeOH-iPrOH (6:1v/v); (□) ACN-iPrOH (6:1v/v); (•) MeOH-ACN-iPrOH (3:3:1v/v). Drawn lines constructed using Eq. (1).

Table 1. Effect of temperature on chromatographic characteristics of clari obtained in a mobile phase containing 55% ACN - iPrOH[a].

Temp. (^{0}C)	Column Pressure (kgf/cm^2)	t_R (min)	Peak Integral (nC)	Peak Height (nA)	Peak Asymmetry	Plate Number	$w_{1/2}$[b] (min)
25	88	9.44	700	32.3	1.32	4423	0.33
30	83	10.17	760	33.1	1.21	4839	0.34
40	75	12.34	949	37.4	1.16	5991	0.37
50	72	14.76	1098	39.6	1.14	6894	0.42
60	61	17.06	1218	42.0	1.06	8276	0.44

[a] injected amount in Intersil column = 0.2 μg.
[b] peak width at half-peak height.

Temperature is one of the important factors, which affects the peak area and shape of the macrolides tested in addition to their retention time. The van't Hoff plots (plots of the natural logarithms of the capacity factors of solutes against the inverse of absolute column temperature) for probe macrolides in different binary, ternary and quaternary eluent systems showed good linearity over the range of temperature from 25 to 70^0 C. However, the sorption enthalpies of these solutes calculated from the slopes of the corresponding regression lines were exothermic (i.e. negative) in all mobile phases, except for the case where ACN or mixture of ACN - iPrOH were used as organic modifiers.

Moreover, higher analysis temperature improved column efficiency for both Clari and Roxi, increased peak area and improved peak shape in all mobile phases used, see Table 1 for the case the ternary eluent system consisting of 55% ACN-iPrOH was used. Therefore, the column temperature is a factor should be taken into account in order to optimise the assay conditions of both macrolides tested.

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