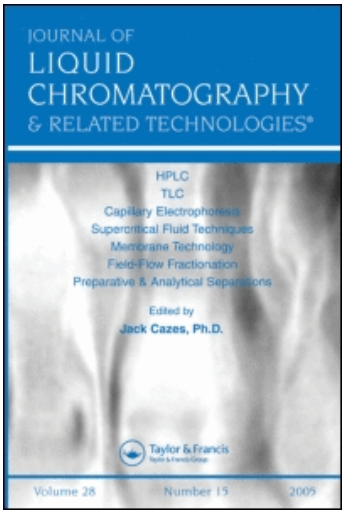

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ION INTERACTION CHROMATOGRAPHY OF CLAVULANIC ACID ON A POLY(STYRENE-DIVINYLBENZENE) ADSORBENT IN THE PRESENCE OF TETRABUTYLAMMONIUM SALTS

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ABSTRACT

The effect of tetrabutylammonium, inorganic coanions, and inert electrolytes, on the clavulanic anion retention on PRP-1 column, formed with 10 μm spherical uniform particles from a polystyrene-divinylbenzene with high pore volume and large surface area, is studied. The results obtained are applied to the chromatographic separation of clavulanic acid from β-lactamic antibiotics and to the determination of clavulanate anion in fermentation broths.

INTRODUCTION

Clavulanic acid (CA) is Z-(2R,5R)-3(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicylo(3,2,0)-heptane-2-carboxylic acid (1). It is a potent inhibitor of β-lactamases (2) which is used mixed with β-lactamase sensitive penicillins to protect them against hydrolysis by the enzyme.

Iodometric and hydroxamic methods frequently used for lactamic antibiotic titration are not appropriate for clavulanic acid quantitative analysis (3).

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Some recent works describing the clavulanic acid spectrophotometric titration by reaction with imidazole (4) have been issued, as well as the determination by HPLC of the derivative obtained (5).

In the present work we have studied the effect of tetrabutylammonium (TBA) salts, inorganic coanions, mixed solvents, and addition of inert electrolytes, on the clavulanic anion retention on PRP-1 column. The PRP-1 column is prepared from polystyrene-divinylbenzene resin in the form of 10 μm spherical uniform particles with high pore volume and large surface area.

MATERIALS AND METHODS

Reagents

Amoxicillin, Cephalexin, and Sodium Ampicillin, were gifts by Antibióticos, S.A. (Madrid, Spain), and they were used as supplied.

Sodium clavulanate was extracted from a pharmaceutical preparation ("Augmentin", Beecham Research Labs., England). Tablets, finely pulverized, were resuspended in cool water and then filtered. The filtrate was extracted at pH 2.5 with n-butanol. The organic phase was extracted again with water, adjusting the mixture to pH 7. The aqueous phase was lyophilized. All these operations were carried out at low temperature (5º C) and as rapidly as possible to avoid the clavulanic acid decomposition. The nature of the product obtained was checked on IR, UV and nmr.

The chemicals were of the highest commercial grade available and were used without further purification. Tetrabutylam-

monium chloride, acetate, formate, propionate, and perchlorate were prepared by passing the tetrabutylammonium bromide solution through Amberlite resin IRA-400 of the forms of chloride, acetate, formate, propionate, and perchlorate, respectively.

Instruments

All the chromatograms were obtained using a modular chromatograph equipped with a Model 6000 A pump, a U6K injector, a Model 441 UV (220 nm) detector, and a strip-chart recorder. All of them manufactured by Waters Ass. A 4.1 mm i.d. x 150 mm, 10 μm PRP-1 (polystyrene-divinylbenzene copolymer) column supplied in a prepacked form by Hamilton Co. was also used.

Chromatographic procedure

The PRP-1 column was conditioned by passing a mobile phase containing tetrabutylammonium salt solution for 1 or 2 hours at 1 ml/min flow rate. When a tetrabutylammonium salt is to be removed the column is first washed by passing al least 20 column volumes of a 50% water-acetonitryle solution through the column and then charged in the desired quaternary ammonium anionic form.

Mixed solvents in the mobile phase are expressed as percent by volume.

Basic mobile phases were prepared from phosphate salts and sodium hydroxide. Ionic strength was controlled when necessary by adding known amounts of inorganic electrolyte.

Sodium clavulanate and amoxicillin were dissolved in the corresponding mobile phases used, injecting 10 μl of these solutions in most cases. The void volume, V_o, was determined from retention volumes found for several samples not retained

on PRP-1 column at the given eluting condition. Depending on these conditions, V_o was about 1.1 ml. All the tests were performed at room temperature and at 1 ml/min flow rate.

RESULTS AND DISCUSSION

The main mobile phase parameters influencing the analyte retention are the nature of the quaternary ammonium salt and its concentration, the nature of the coanion, pH, type and concentration of mixed solvents, and ionic strength. Though these parameters are generally related, the adjustment of the three first ones are usually most critic. In our case, the working pH has always been between 6 and 7 due to the instability of the clavulanic acid at pH different from neutrality (6).

Effect of R_4N^+ concentration

In order to carry out the present study, the retention times of clavulanate and amoxicillin on a PRP-1 column were measured using mobile phases formed by 0.001 M phosphate buffer at pH 6 and acetonitryle (9-1 v/v) containing tetrabutylammonium bromide at different concentrations. The results obtained are given in Fig. 1.

In a system formed by a nonpolar phase of polystyrene-divinylbenzene copolymer, and mobile phases formed by solvents in which water predominates and which contains R_4N^+ cations and their corresponding coanions, the procedure that determines the chromatographic separation is probably based on a series of equilibria in which anion exchanges predominate. ISKANDARINI and PIETRZYK (7) have deduced Equation 1 from the study of major equilibria that contribute to an organic analyte

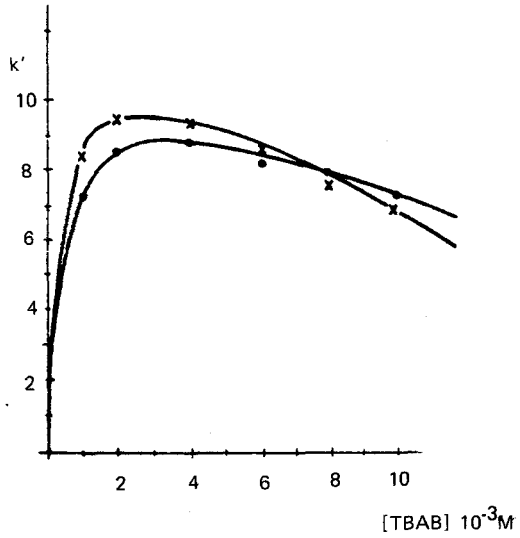

Figure 1. Effect of tetrabutylammonium bromide concentration on the capacity factor of sodium clavulanate (•) and amoxicillin (x) on PRP-1 column. The mobile phase conditions are 1:9 $CH_3CN:H_2O$ 1.0 mM phosphate buffer pH 6 and variable TBAB concentrations.

anion retention, under conditions similar to those experienced by us. Such equation expresses the capacity factors in function of a series of parameters.

$$\frac{1}{k'} = \frac{1}{qK_oK(R_4NXA)_s} \cdot \frac{1}{(R_4N^+)_m} + \frac{K(R_4NCA)_s}{qK_oK(R_4NXA)_s} \cdot \left[C^-\right]_m +$$

$$+ \frac{1}{qK_o}\left[1 + \frac{K_{R_4NX} \cdot K(R_4NCA)_s}{K(R_4NXA)_s}\right]\left[X^-\right]_m \qquad \text{(Eq. 1)}$$

where: k' is the capacity factor for a determined analyte.

$[R_4N^+]_m$ $[C^-]_m$ and $[X^-]_m$ are the concentrations for the mobile phase of quaternary ammonium salt, of coanion and of analyte anion, respectively. "q" is the ratio of stationary phase volume to mobile phase volume. K_o is the sorption capacity for the stationary phase and a measure of the number of sites that can be occupied in the retention process.

$K_{(R_4NXA)_s}$ and $K_{(R_4NCA)_s}$ are the retention equilibrium constants of R_4NX and R_4NC on the stationary phase. X represents the analytic anion and C the R_4N^+ coanion.

K_{R_4NX} is the equilibrium constant for the anion exchange:

$$(R_4NCA)_s + [X^-]_m \rightleftharpoons (R_4NXA)_s + [C^-]_m$$

We have experimentally found (Fig. 1) that on increasing R_4N^+ concentration the values of the capacity factors of the analyte anion also increase, reaching an optimum value from which they begin to increase. Equation 1 describes this phenomenon at least qualitatively. In fact, on increasing R_4N^+, its coanion C^- concentration also increases at a same ratio. The coanion exerts a competitive action with the analyte anion adsorption, expressed in Equation 1 by the second number to be added. On increasing the coanion concentration, the second number to be added predominates on the first one in Equation 1; this justifies the presence of a maximum in Fig. 1

Effect of R_4N^+ coanion

Few works have studied the effect of the nature of anions in solution or accompanying the R_4N^+ salt on the retention of the organic anionic analytes.

Table 1 shows k´ values determined by amoxicillin and clavulanic acid retention on PRP-1 column in the presence of tetrabu-

TABLE 1

Effect of Tetrabutylammonium Coanions on Amoxicillin and Sodium Clavulanate Retention on PRP-1 Column.

	Capacity factors k′							
	$Butyr^-$	$Prop^-$	$Acet^-$	$Form^-$	$ClO4^-$	$SO_4^=$	Cl^-	Br^-
Clavulanate	3.5	5.1	12.4	6.4	0.54	3.6	4.7	5.7
Amoxicillin	2.7	7.0	34.8	14.9	0.81	3.4	4.8	3.6

tylammonium and different types of coanions, at an ionic strength of 0.01 completed with the sodium salt of the corresponding coanion, always at pH between 6 and 7. At this pH, both clavulanic acid and amoxicillin are in the anion form.

As it can be seen in Table 1, the nature of coanions exerts a remarkable effect on the clavulanate and amoxicillin anion retention. However, this effect is not the same on amoxicillin as on clavulanic acid. The order of the effect of different coanions, from maximum to minimum retention for amoxicillin is as follows:

$$Ac^- > Form^- > Prop^- > Cl^- > Br^- > SO_4^= > Butyr^- > ClO_4^-$$

while the order for clavulanate is:

$$Ac^- > Form^- > Br^- > Prop^- > Cl^- > SO_4^= > Butyr^- > ClO_4^-$$

Given the composition of the mobile phase used, it is probable that the phenomenon which rules the clavulanate ion retention will be an anion exchange. However, we must stand out that the experimental verification of Eq. 1 does not prove that the process is necessarily an anion exchange, since Equation 1 can also be derived where only ion pairs formation between R_4N^+ and the different anion is considered. In the latter case, the

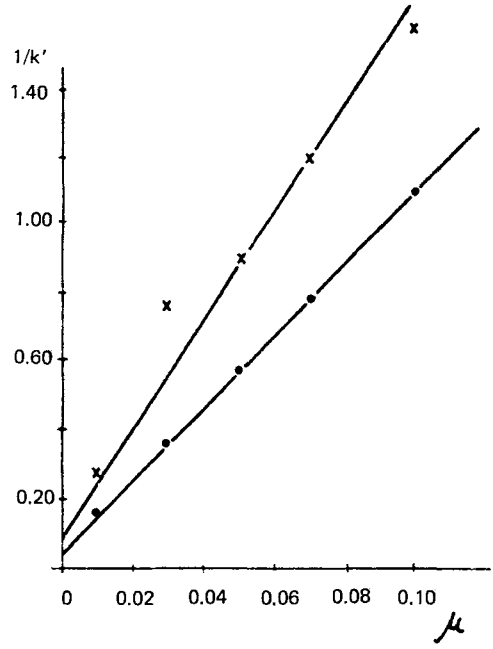

Figure 2. Effect of ionic strength on amoxicillin (x) and clavulanate ion (•) retention. Column: PRP-1. Mobile phase: (1-9 v/v.) acetonitryle 1.0 mM phosphate buffer pH 6, 3.0 mM TBAB and variable ionic strength (NaBr).

equilibrium constant in Equation 1 would be the ion pairs constants. We think ion pairs formation will probably play a more important role for mobile phases having a high organic solvent/water ratio, which does not occur in our case.

Effect of ionic strength

The effect of ionic strength in mobile phase, on clavulanic acid and amoxicillin retention on PRP-1 column, with a mobile phase formed by 0.001 M phosphate buffer, pH 6-acetonitryle (9-1 v/v) which is at the same time 0.03 M in tetrabutylammonium bromide at an ionic strength between 0.01 and 0.1 set with NaBr,

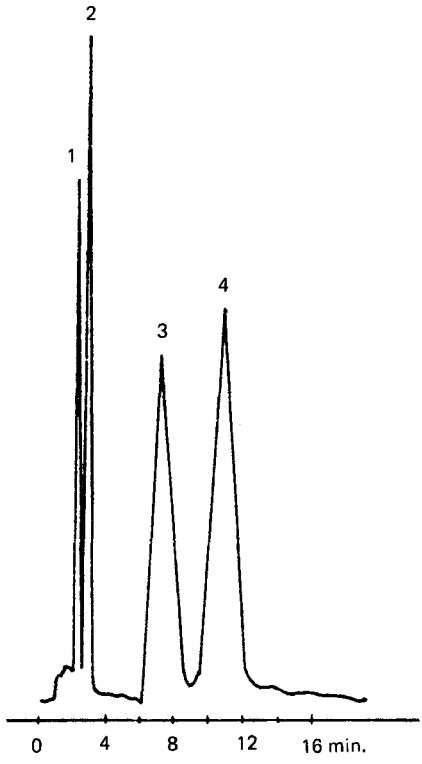

Figure 3. Column, 150 x 4.1 mm i.d., 10 μm PRP-1. The mobile phase conditions are 1-9 acetonitryle-1.0-mM phosphate buffer pH 6, 3.0 mM TBAB, μ= 0.05 (NaBr). Flow rate, 1.0 ml/min U.V. detector at 230 nm. 1 Amoxicillin, 2 Sodium clavulanate, 3 Cephalexin, 4 Sodium Ampicillin.

is shown in Fig. 2. As the ionic strength increases, $1/k'$ also increases, resulting in a linear ratio as can be predicted from Equation 1.

All the experimental results seem to suggest that clavulanic acid and amoxicillin retention on PRP-1 column, in the presence of tetrabutylammonium salt, is the results of a complex set of equilibrium, taking place within a double layer at

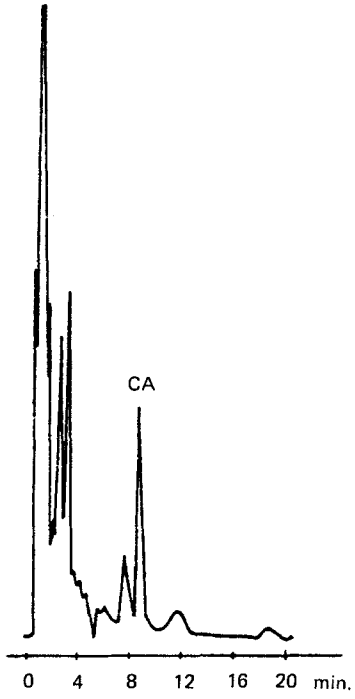

Figure 4. Column, 150 x 4.1 mm i.d., 10 μm PRP-1. The mobile phase conditions are 1-9 acetonitryle-1.0-mM phosphate buffer pH 6, 3.0 mM TBAB. Flow rate, 1.0 ml/min. Sample: Streptomyces clavuligerus ATCC 27064 fermentation broth.

the stationary phase surface. The coanion and/or anion of the buffer would also contribute on the second layer and would be a part of a competitive equilibrium.

Applications

The above shown may be applied to the chromatographic separation of clavulanic acid in different mixtures. Thus, for example, Fig. 3 illustrates the separation of a mixture of clavulanic acid, amoxicillin, cephalexin, and ampicillin using a PRP-1 column and a mobile phase formed by (1-9 v/v) aceto-

nitryle-10 mM phosphate, pH 6, in the presence of 3.0 mM TBAB and $\mu = 0.05$ (NaBr).

Fig. 4 shows an example of clavulanic acid separation in a Streptomyces clavuligerus ATCC 27064 fermentation broth, using a mobile phase (1-9 v/v) acetonitryle-1.0 mM phosphate solution, pH 6, containing 3.0 mM TBAB.

REFERENCES

1. Howarth, T.T., Brown, A.G., and King, T.J., J. Chem. Soc. Chem. Commun., 266, 1976.

2. Reading, C., and Cole, M., Antimicrob. Agents Chemother., 11, 852, 1977.

3. Charnas, R.L., Fisher J., and Knowles, J.R., Biochemistry, 17, 2185, 1978.

4. Bird, A.E., Bellis, J.M., and Gasson, B.C., Analyst, 107 1241, 1982.

5. Foulstone, M., and Reading, C., Antimicrob. Agents, Chemother., 22, 753, 1982.

6. Haginaka, J., Nakagawa, T., and Uno, T., Chem. Pharm. Bull, 29/11, 3334, 1981.

7. Iskandarini, Z., and Pyetryzk, D.J., Anal. Chem., 54, 1065, 1982.

8. Rotsch, T.D., Cahill, W.R., Pietrzyk, D.J., and Cantwell, F.F., Can. J. Chem., 59, 2179, 1981.

9. Rotsch, T.D., and Pietrzyk, D.J., J. Chromatogr. Sci., 19, 88, 1981.