HROM. 4234

artition data of cephalosporins determined by means of reversed-phase hin-layer chromatography

In a previous paper a reversed-phase thin-layer chromatography method was hown to be a suitable technique for the determination of partition data of penicillins[1].

The reversed-phase TLC method previously described and discussed[1] is used in he present work for the determination of partition data of cephalosporins. Silicone il was used as the stationary phase. An aqueous mobile phase was used (sodium cetate veronal buffer at pH 7.4) alone or in various proportions with acetone. The tructures of the tested cephalosporins are indicated in Table I. The spotting and .etection of the compounds were performed as previously described[1].

Results and discussion

The cephalosporins migrated practically without tailing as round spots as can ›e seen in Fig. 1. It is interesting to note that the iodine azide solution, suitable for ›enicillins, was ineffective in detecting the cephalosporins. The transformation of R_F values into R_M values permitted one to obtain a series of data where positive and ıegative R_M values derive from R_F values respectively smaller and greater than 0.5. Therefore higher and/or positive R_M values indicate compounds more lipophilic than :hose represented by a lower and/or negative R_M value. The plots of the R_M values versus the composition of the mobile phase are represented in Fig. 2. For each com›ound there was a range of linear relationship between R_M values and composition of :he mobile phase. The straight lines of Fig. 2 were calculated by means of the least-ıquare method from the R_M values in the range of linearity. Therefore only the R_M values corresponding to acetone concentrations between 0 and 10–12–16% were used. At higher concentrations the R_M values started to show the tendency of the com›ounds to migrate with the solvent front. For the lowest (most hydrophilic) compound

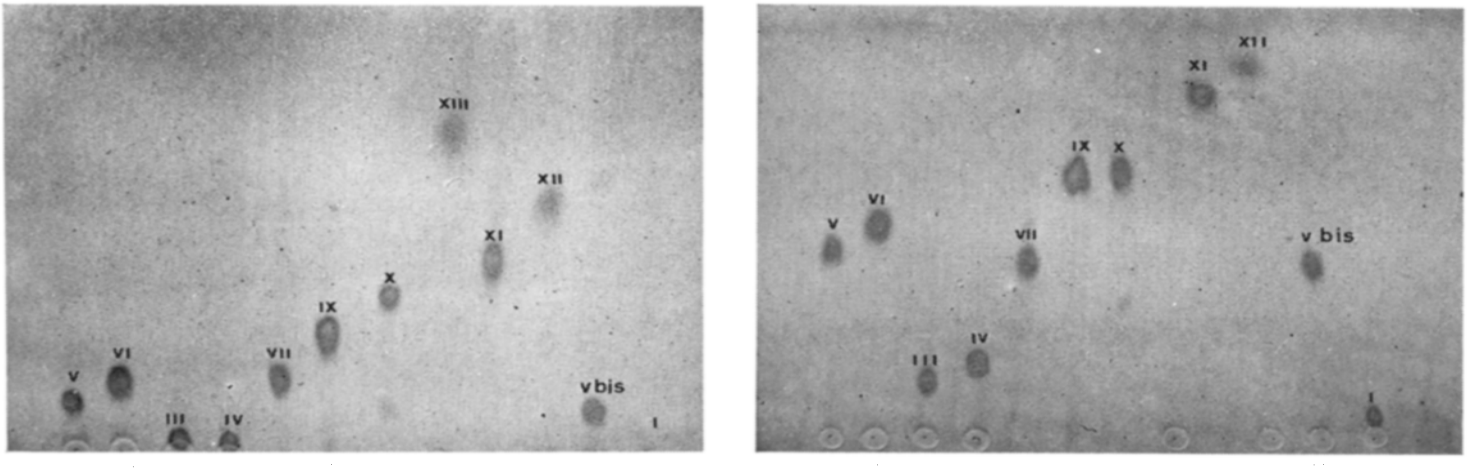

Fig. 1. Reversed-phase TLC of some cephalosporins. Stationary phase: silicone oil on Silica Gel G layer. Mobile phase: buffer in (a) and acetone-buffer 10% in (b). Detection: potassium permanganate alkaline solution. Amounts: 1 μg of each compound. The compounds are indicated as in Table I. Compound V bis corresponds to acid Cephaloram. In (b) compound XIII is no longer detectable as it migrated with the solvent front.

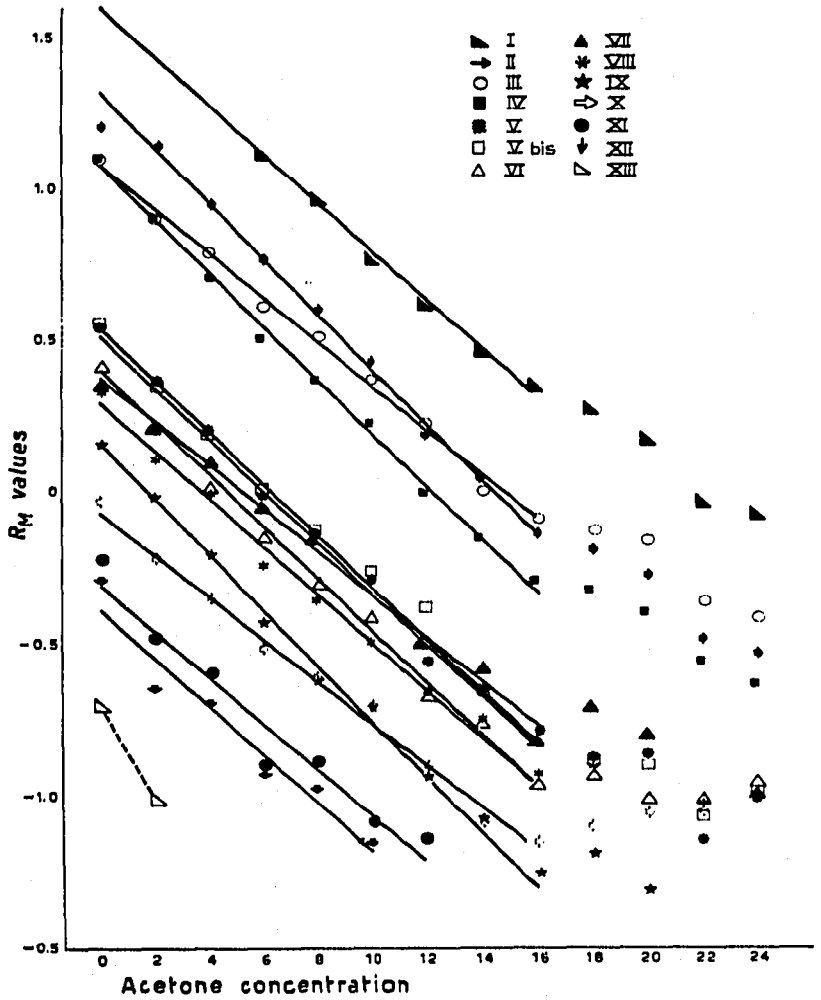

Fig. 2. The R_M values are plotted against the composition of the mobile phase. The straight lines were calculated by means of the least-squares method, except in the case of the lowest compound. Here the points were connected by a dotted line. Each point represents the mean of eight determinations. The R_M values corresponding to acetone concentrations higher than 24% are not reported. The cephalosporins are indicated as in Table I. Compound V bis corresponds to acid Cephaloram.

there were only two available points because with higher acetone concentrations in the mobile phase it migrated with the solvent front. In Fig. 2 these two points were simply connected by a dotted line. The R_M value indicated in Table I is therefore the experimental R_M value with buffer as the mobile phase. The highest (most lipophilic) compound I did not move until a certain acetone concentration was reached. By means of the equations of the straight lines of Fig. 2 the interpolated or extrapolated R_M values at 0% were calculated. In this way it was possible to obtain, for each compound, a R_M value in a standard system of water–silicone oil.

The calculated R_M values are reported in Table I. They permit the study of the influence of substituent groups on the partitioning of cephalosporins. The lipophilic character of compound II is more and more decreased by the substitution of the naphthyl moiety in the side chain with a benzene, a thiophene or a furan ring, as in compounds V, VI and IX respectively. The hydrophilic character of compound V is

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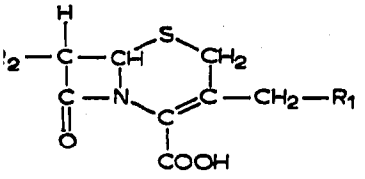
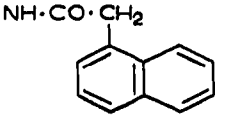
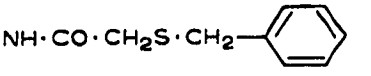
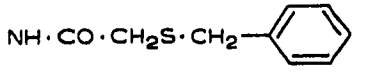
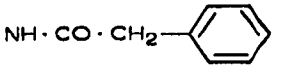
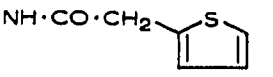
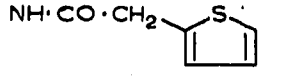
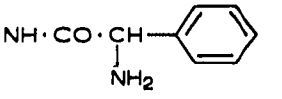
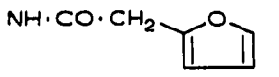
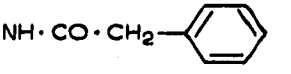
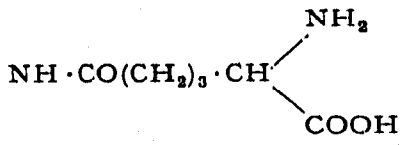
ST OF THE CEPHALOSPORINS ACCORDING TO THE DECREASING LIPOPHILIC CHARACTER OF THEIR OLECULES, AS EXPRESSED BY THEIR CALCULATED R_M VALUES

he experiments were carried out with the sodium and potassium salts or with the free acids. ephaloram was used both as sodium salt and free acid.



ompounds	R_1	R_2	R_M *value*
(acid)	$O\cdot CO\cdot CH_3$	$NH\cdot CO\cdot (CH_2)_6\cdot CH_3$	1.60
[(acid)	$O\cdot CO\cdot CH_3$		1.32
II (sodium salt)	N_3		1.08
V (sodium salt)	$O\cdot CO\cdot CH_3$		1.08
(sodium Cephaloram)	$O\cdot CO\cdot CH_3$		0.54
I (sodium Cephalotin)	$O\cdot CO\cdot CH_3$		0.40
II (acid)	N_3		0.37
III (acid Cephaloglycin)	$O\cdot CO\cdot CH_3$		0.29
X (acid)	$O\cdot CO\cdot CH_3$		0.16
(acid)	OH		−0.07
XI (acid)	$O\cdot CO\cdot CH_3$	$NH\cdot CO\cdot CH_2Cl$	−0.31
XII (7-amino-cephalosporanic acid)	$O\cdot CO\cdot CH_3$	NH_3^+	−0.39
XIII (potassium Cephalosporin C)	$O\cdot CO\cdot CH_3$		−0.71

increased by the introduction in the side chain, as in compound VIII, of a NH_2 group or by the substitution of the $OCOCH_3$ group with an OH one, as in compound X, or of the benzene ring with a Cl atom, as in compound XI. The substitution of a $OCOCH_3$ group with a N_3 does not seem to influence the R_M value in a significant way. This was noted for compounds IV and III and for VI and VII, respectively. However at higher acetone concentrations the compounds with the N_3 group seem to be less hydrophilic than those with the $OCOCH_3$ group.

The data of the present work confirm the existence of a linear relationship between R_M values and composition of the mobile phase. This relationship, first noted by SOCZEWINSKI AND WACHTMEISTER[2], is useful because it permits the calculation, from the range of maximum accuracy, of the R_M values for all the compounds in a standard system. It can be noted that the acidic form and the sodium salt of Cephaloram practically give the same results. This was also observed in the case of penicillins[1,3]. The considerations about the influence of the substituents on the partitioning of cephalosporins, as indicated by their R_M values, permit conclusions to be drawn which are in agreement with those of other investigators. The lipophilic character of the naphthyl substitution in comparison with a benzene and a thiophene ring was shown by HANSCH *et al.*[4] and IWASA *et al.*[5]. The hydrophilic character of the substitution of a H atom, an $OCOCH_3$ group and a benzene ring respectively with an NH_2, an OH and a Cl atom was also shown by IWASA *et al.*[5].

It can be noted that the iodine–azide solution, which is effective in detecting phenylthiohydantoins[6], thiophosphoric esters[7] and penicillins[3], did not succeed in detecting the spots of cephalosporins.

The advantages of the present method and its suitability for studying the relationship between partition data and biological activity of drugs have already been pointed out[1].

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