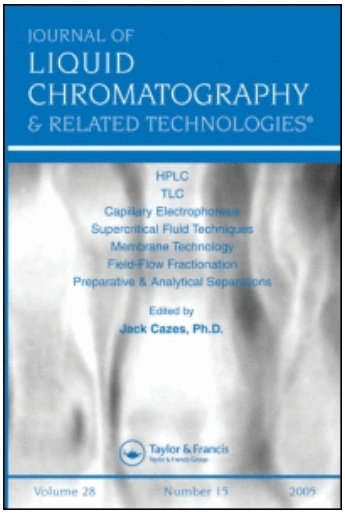

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TLC Separation of Cephalosporins: Searching for Better Selectivity

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TLC Separation of Cephalosporins: Searching for Better Selectivity

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Abstract: Cephalosporins are broad-spectrum bactericidal cell wall antibiotics which are very similar in structure and action to penicillins. They are used mainly for treating staphylococcal and streptococcal infections in patients who cannot use penicillins. Cephalosporins are also often used in veterinary medicine. They are commonly divided into four groups which differ slightly in spectrum and toxicity. Cephalosporins can be detected in pharmaceuticals, body fluids, and food by microbiological and immunological assays, capillary electrophoresis, chromatography, and other physico-chemical methods. In this paper, the optimal conditions for thin-layer chromatographic analysis of eight cephalosporins, i.e., cefaclor, cefoperazone, cefazolin, cefotaxime, cefoxitin, cefuroxime, cephalotin, and p-chlorophenacyl cephalothin ester were established. Retention parameters for various chromatographic systems were compared. Good separations were achieved for several phases on plane silica gel and silica gel with boned diol-, amino- and cyanopropyl chains.

Keywords: HPTLC, Cephalosporins, Silica, Diol-silica, Amino-silica, Cyano-silica

INTRODUCTION

Cephalosporins are derived from natural cephalosporin C produced by the *Cephalosporium acremonium* fungus. Structurally they are closely related to penicillins and exhibit the same mechanisms of action, i.e., they inhibit bacterial cell wall synthesis of both Gram-positive and Gram-negative bacteria. However, they are much safer for patients than penicillins.

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Cephalosporins are also often used in veterinary medicine, mainly for curing mastitis in cows.

The most popular methods for cephalosporin analysis are microbiological assays,[1–3] high performance liquid chromatography (HPLC),[4–8] and thin-layer chromatography (TLC).[9–17]

Cephalosporins can be analyzed both by normal and reverse phase TLC. The most popular adsorbent for normal phase (NP) systems is silica gel. Silica can be also impregnated with Na_2EDTA, tricaprylmethylammonium chloride, and transition metal ions. Inorganic ion-exchangers and silica gel mixed with an exchanger can be also used as stationary phases. Mobile phases used in analysis of cephalosporins are polar and are similar to those used for penicillins. All cephalosporins can be detected at 254 nm and by bioautography with, for instance, *Neisseria catarrhalis*. Applying reagents such as ninhydrin, potassium iodoplatinate, Dragendorff's reagent, sulfuric acid, and iodine vapor can diminish the detection limit. Some examples of TLC separation are given below.

Misztal et al.[11] developed solvent systems, both NP and reverse phase (RP), for the separation of 7 selected cephalosporins. The best NP solvents could separate up to six analyzed drugs. All cephalosporins were detected at 254 nm. Eight modes of detection, besides UV at 254 nm, were described. Quintens et al.[10] described the procedure that enables identification of 30 cephalosporins on TLC silanized silica gel plates containing a fluorescence indicator. The mobile phases were composed of a buffer mixed with organic modifiers such as methanol, acetonitrile, acetone, tetrahydrofuran, ethanol, or methyl acetate. No system separated all the cephalosporins, but they could be identified when supplementary information was obtained from color reactions and/or additional TLC systems. Dhenasar[16,17] described the use of scanning densitometry for direct quantitation of ceftriaxone and twelve other cephalosporins on hydrocarbon-impregnated silica gel plates without prior solvent elution.

Recently, Tuzimski[9] separated eight cephalosporins by two-dimensional TLC on silica gel layers. The best selectivity was obtained by use of the methanol-98% formic acid-ethyl acetate (29:1:70) in the first direction and methanol-toluene-ethyl acetate-98% formic acid (5:20:65:10) in the second direction.

In this paper, the optimal conditions for the separation of the eight cephalosporins on plane silica gel and silica gel with bonded diol-, amino-, and cyanopropyl chains were established.

EXPERIMENTAL

Equipment and Reagents

DS sandwich chambers[18] were purchased from Chromdes, Lublin, Poland. Precoated HPTLC plates: $Si60F_{254}$, Diol F_{254}, CN F_{254} and NH_2 F_{254}

10 cm × 10 cm were purchased from E. Merck, Darmstadt, Germany. Methanol, 2-propanol, diisopropyl ether, toluene, ethyl acetate, 80% formic acid, and citric acid, analytical grade, were purchased from P.O.Ch. Gliwice, Poland. The cephalosporins were the gift of Dr. Tuzimski (Medical University, Lublin). They are numbered from 1 to 8, i.e, cefaclor – 1; cefoperazone sodium – 2; cefazolin sodium – 3; cefotaxime sodium – 4, cefoxitin sodium – 5, cefuroxime sodium – 6, cephalotin sodium – 7, p-chlorophenacyl cephalothin ester – 8. Their structures are given in paper.[9]

Methods

Cephalosporin working solutions were prepared in methanol (except for p-chlorophenacyl cephalotin ester which was dissolved in acetone) as 1 mg mL^{-1} standards. The mixture of these antibiotics was prepared in methanol at 1 mg mL^{-1} of each.

The standards of the cephalosporins were applied to the TLC plates as 1 μL spots using a Hamilton microsyringe (Bonaduz, Switzerland). After air drying, the spots of the antibiotics were detected at 254 nm by UV lamp (HA-05 Haland, Warsaw, Poland) or by Camag Reprostar 3 Video Camera (Muttenz, Switzerland).

RESULTS AND DISCUSSION

The following mobile phases, denoted by capital letters, were tested for the separation of the eight cephalosporins:

A	methanol-ethyl acetate-80% formic acid (29:70:1)
B	methanol-toluene-ethyl acetate-80% formic acid (5:20:65:10)
C	methanol-toluene-ethyl acetate-80% formic acid (4:30:65:1)
D	2-propanol-toluene-ethyl acetate-80% formic acid (4:30:65:1)
E	2-propanol-ethyl acetate-80% formic acid (29:70:1)
F	methanol-water-80% formic acid (80:19:1)
M	diisopropyl ether-toluene-ethyl acetate-80% formic acid (4:30:65:1)
Q*	methanol-toluene-ethyl acetate-80% formic acid-water (5:20:65:1:9)
Q/B	Q-B (1:1)
R*	methanol-toluene-ethyl acetate-0.01M citric acid (5:20:65:10)
U	2-propanol-ethyl acetate-80% formic acid (25:70:5)
V	methanol-toluene-ethyl acetate-80% formic acid (4:30:65:5)
W	2-propanol-ethyl acetate-80% formic acid (19:80:1)

X	2-propanol-toluene-ethyl acetate-80% formic acid (5:20:65:10)
Y	diisopropyl ether-toluene-ethyl acetate-80% formic acid (5:20:65:10)
Z	methanol-toluene-ethyl acetate-80% formic acid (5:20:65:1)
XX*	methanol-toluene-ethyl acetate-0.1 M citric acid-80% formic acid (5:20:65:5:5)
XY	2-propanol-ethyl acetate (30:70)
AA	diisopropyl ether-ethyl acetate-80% formic acid (29:70:1)

The solvents A and B were used by Tuzimski[9] for 2D TLC of the cephalosporins. For the solvents denoted by asterisks (demixed because of too high water content), the upper layers were used.

The R_f and R_m values, retention factors k, and separation factors α for the pairs of cephalosporins were calculated. Tables 1–4 contain hR_f values for cephalosporins developed on plane silica, diol-, amino-, and cyano-silica for different solvents.

Figures 1 to 7 present correlation plots between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on various bonded phases, i.e., Diol, CN and NH_2 for the solvents A, B, C, D, E, M, and Q, respectively. It can be stated that amino plates are for most systems too polar and retained

Table 1. The hR_f values obtained for cephalosporins (numbered from 1 to 8) for various phases on HPTLC SiO_2 F_{254} plates

Mobile phase	hR_f 1	hR_f 2	hR_f 3	hR_f 4	hR_f 5	hR_f 6	hR_f 7	hR_f 8
A	11	27	41	48	54	59	71	99
B	12	17	30	24	55	59	73	90
C	0	0	3	5	9	12	27	86
D	0	0	2	5	10	12	27	89
E	0	14	23	36	48	55	66	98
M	0	1	3	4	11	14	30	84
Q	0	0	5	7	9	13	25	99
Q/B (1:1)	0	5	13	15	28	34	53	88
R	0	0	0	0	0	0	0	88
U	10	30	49	57	76	79	90	99
V	1	3	14	13	29	34	53	86
X	9	12	25	20	55	60	76	92
Y	5	6	15	12	41	47	66	89
XY	0	0	0	0	0	0	2	0
XX	2	8	15	14	30	35	56	87
AA	0	0	1	3	7	9	21	94

Table 2. hR_f values obtained for cephalosporins (numbered from 1 to 8) for various phases on HPTLC Diol F_{254} plates

Mobile phase	hR_f 1	hR_f 2	hR_f 3	hR_f 4	hR_f 5	hR_f 6	hR_f 7	hR_f 8
A	83	84	85	87	88	88	94	98
B	28	53	59	55	73	76	85	95
C	3	16	29	30	38	41	66	91
D	4	23	36	40	53	55	75	90
E	0	83	84	88	92	93	98	100
M	0	5	15	16	25	29	58	88
Q	6	39	41	41	49	51	73	94
W	21	71	77	80	85	87	94	99
X	27	69	59	54	76	78	88	95

Table 3. hR_f values obtained for cephalosporins (numbered from 1 to 8) for various phases on HPTLC CN F_{254} plates

Mobile phase	hR_f 1	hR_f 2	hR_f 3	hR_f 4	hR_f 5	hR_f 6	hR_f 7	hR_f 8
A	31	96	96	96	98	98	98	98
B	34	74	74	61	93	93	98	98
C	4	46	50	43	75	80	86	94
D	0	45	57	45	78	81	88	95
E	3	93	93	89	—	—	—	—
M	0	21	38	26	67	73	84	95
Q	0	41	55	46	78	82	87	98
F	89	93	93	94	94	94	90	61
V	0	69	59	49	82	85	89	95
Z	2	49	59	52	79	84	88	94

Table 4. hR_f values obtained for cephalosporins (numbered from 1 to 8) for various phases on HPTLC NH_2 F_{254} plates

Mobile phase	hR_f 1	hR_f 2	hR_f 3	hR_f 4	hR_f 5	HR_f 6	hR_f 7	hR_f 8
A	0	16	16	0	0	16	15	15
B	4	6	14	20	21	26	49	50
C	0	0	0	1	0	0	1	0
D	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0
Q	0	0	0	0	0	0	0	0

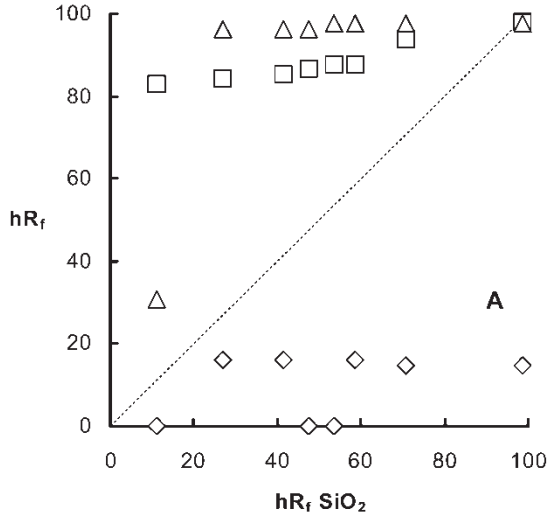

Figure 1. Correlation between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent A. The dashed line denotes the relation $y = x$.

the drugs too strongly. By contrast, cyano plates are in most cases too low in polarity for good separation of cephalosporins. Diol-silica has the most similar retention properties to silica gel. As can be seen, there are only a few systems which can separate all the analyzed antibiotics, but many systems give a nice

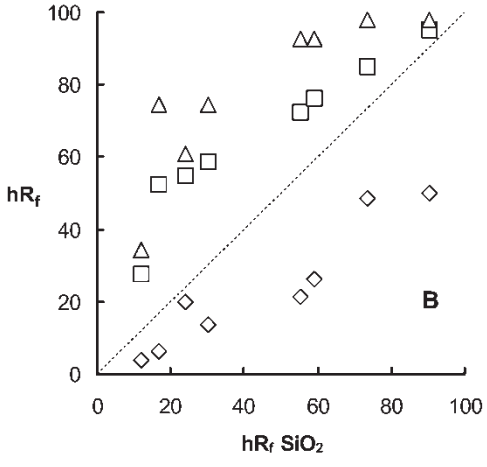

Figure 2. Correlation between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent B.

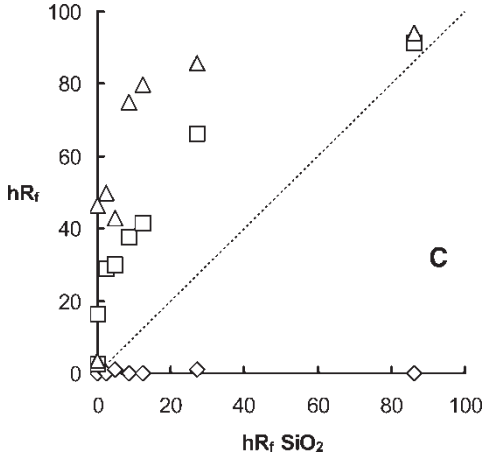

Figure 3. Correlation between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent C.

separation of almost all of them. The separation factors obtained for the systems separating all the drugs are presented in Table 5. The systems which provide good α values for the pairs of antibiotics, but with spots that are tailing (e.g., SiO_2/E), are not included in the Table. The chromatogram

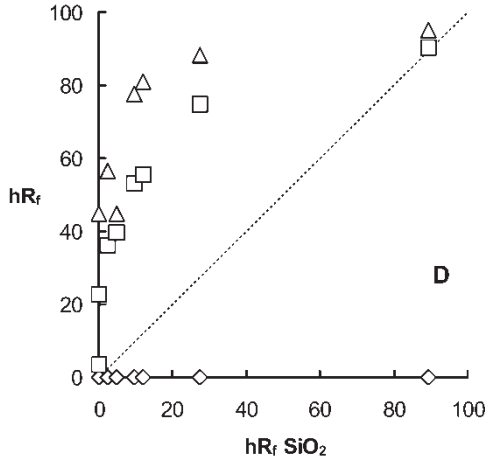

Figure 4. Correlation between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent D.

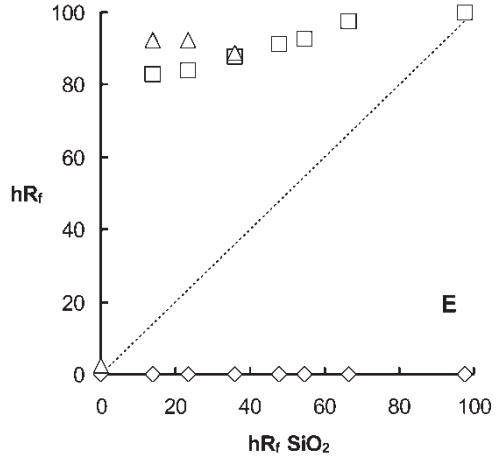

Figure 5. Correlation between hR_f values obtained on HPTLC silica gel plates and hR values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent E.

for one of the systems presented in the Table, i.e., the mobile phase Y and silica gel is shown in Fig. 8.

Figure 9 presents hR_f values of eight cephalosporins obtained on silica gel for three mobile phases of similar composition. They differ only in the

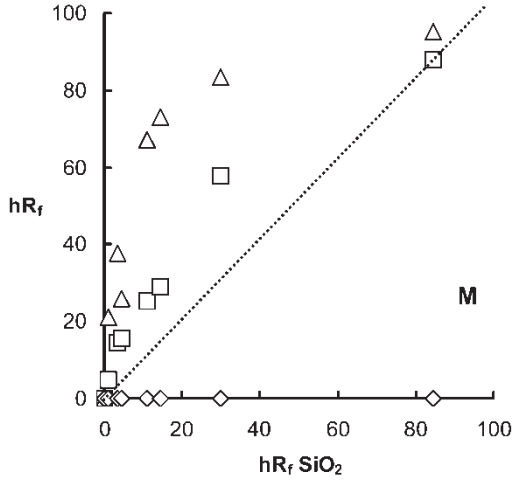

Figure 6. Correlation between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent M.

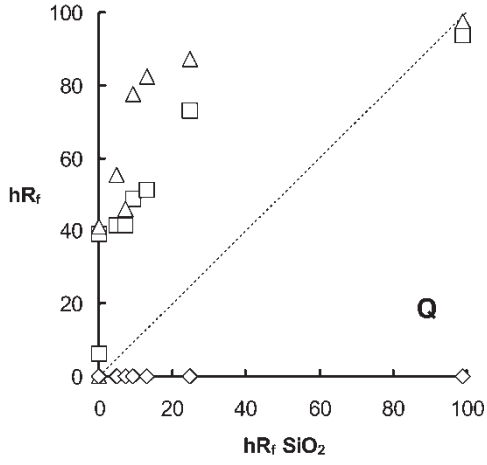

Figure 7. Correlation between hR_f values obtained on HPTLC silica gel plates and hR_f values obtained on HPTLC amino-silica (rhombus), HPTLC diol-silica (square) and HPTLC cyano-silica (triangle) for the solvent Q.

kind of the first component. The phase C contains methanol, D 2-propanol and M diisopropyl ether. This is no influence of the modifier type on the hR_f values probably because of too low elution strength of the solvent (a small formic acid content). Figures 10 and 11 show similar dependencies obtained for two other sets of mobile phases on silica gel. For the phase 5:20:65:10 (modifier-toluene-ethyl acetate-80% formic acid) with higher content of formic acid and for the phase 29:70:1 (modifier-ethyl acetate-80% formic acid) the following relation can be observed: the higher the polarity of the first component, the higher the hR_f values. This is especially evident for the phases 29:70:1 with high content of the modifier. The phases

Table 5. Separation factors for the pairs of cephalosporins for various systems. In brackets $\alpha_{4/3}$ values (in these systems the elution order for the pairs 3/4 is reversed)

	SiO_2/A	SiO_2/B	SiO_2/Y	Diol/C	Diol/D	CN/M	CN/Q
$\alpha_{1/2}$	2.97	1.48	1.27	7,59	7,95	—	—
$\alpha_{2/3}$	1.93	2.12	2.89	2,08	1,90	2,24	1,77
$\alpha_{3/4}$	1.28	0.74 (1.35)	0.74 (1.35)	1,06	1,17	0,58 (1.72)	0,69 (1.45)
$\alpha_{4/5}$	1.28	3.92	5.25	1,40	1,71	5,84	4,10
$\alpha_{5/6}$	1.22	1.16	1.27	1,17	1,10	1,32	1,34
$\alpha_{6/7}$	1.71	1.92	2.17	2,80	2,38	1,88	1,44
$\alpha_{7/8}$	33.52	3.38	4.37	5,33	3,19	4,01	6,17

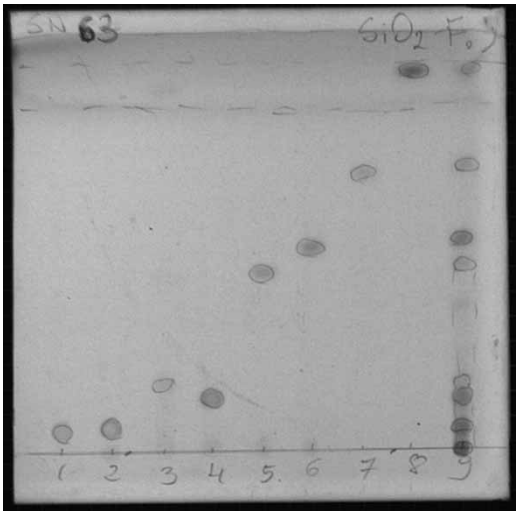

Figure 8. Video image of the chromatogram of eight cephalosporins and their mixture on HPTLC silica gel F_{254} plate for the phase Y.

C, D, M which give very similar separations on silica gel provide different separations of cephalosporins on diol and CN plates (Figs. 12 and 13). On diol-silica plates, 2-propanol gives the highest hR_f values, the second is methanol and the weakest elution strength has diisopropyl ether. The

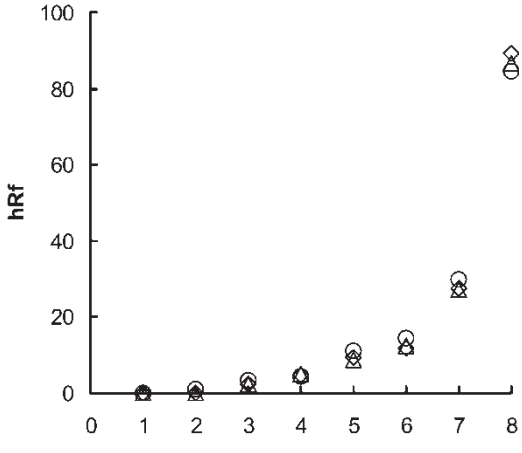

Figure 9. hR_f values of eight cephalosporins obtained on silica gel for three mobile phases of general composition: modifier-toluene-ethyl acetate-80% formic acid (4:30:65:1). Triangle – methanol is the modifier (the phase C), rhombus – 2-propanol (the phase D), circle – diisopropyl ether (the phase M).

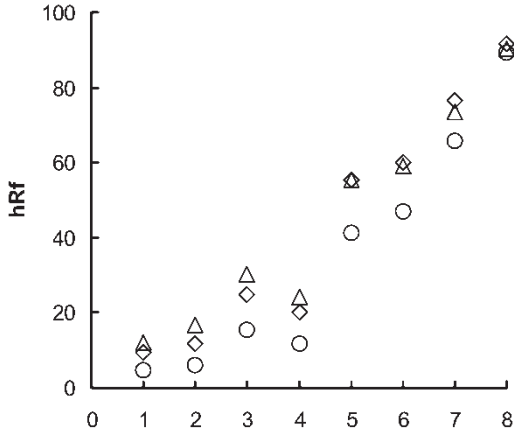

Figure 10. hR_f values of eight cephalosporins obtained on silica gel for three mobile phases of general composition: modifier-toluene-ethyl acetate-80% formic acid (5:20:65:10). Triangle – methanol is the modifier (the phase B), rhombus – 2-propanol (the phase X), circle – diisopropyl ether (the phase Y).

solvents C, D, M are not suitable for the separation on amino-silica plates, which retains cephalosporins too strongly. Figure 14 compares the phases with various contents of an acid and/or water. There is no elution on silica gel when the phase contains water or aqueous solutions of citric

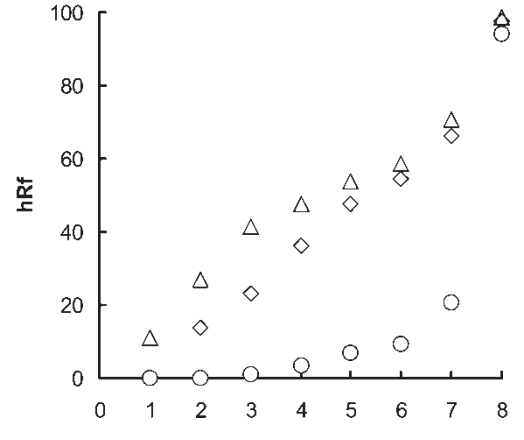

Figure 11. hR_f values of eight cephalosporins obtained on silica gel for three mobile phases of general composition: modifier-ethyl acetate-80% formic acid (29:70:1). Triangle – methanol is the modifier (the phase A), rhombus – 2-propanol (the phase E), circle – diisopropyl ether (the phase AA).

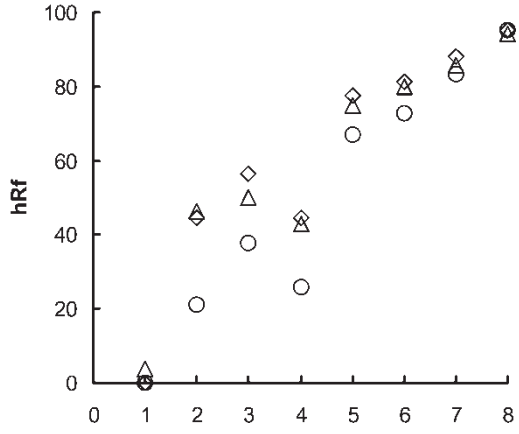

Figure 12. hR_f values of eight cephalosporins obtained on cyano-silica for three mobile phases of general composition: modifier-toluene-ethyl acetate-80% formic acid (4:30:65:1). Triangle – methanol is the modifier (the phase C), rhombus – 2-propanol (the phase D), circle – diisopropyl ether (the phase M).

acid instead of formic acid. The phase Q which has the proportion of formic acid to water 1:9 gives hR_f values in the range 0–25, despite p-chlorophenacyl cephalothin ester, which is not retained. The phases which contain formic acid in a volume above 5 elute the drugs better

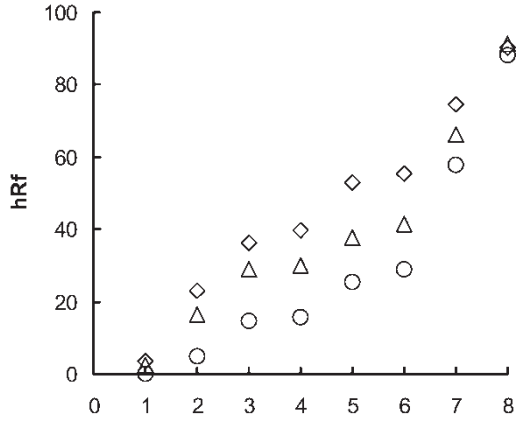

Figure 13. hR_f values of eight cephalosporins obtained on diol-silica for three mobile phases of general composition: modifier-toluene-ethyl acetate-80% formic acid (4:30:65:1). Triangle – methanol is the modifier (the phase C), rhombus – 2-propanol (the phase D), circle – diisopropyl ether (the phase M).

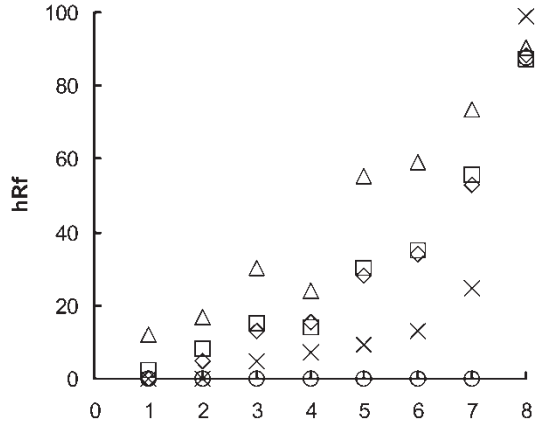

Figure 14. hR_f values of eight cephalosporins obtained on silica gel for five mobile phases of general composition: methanol-toluene-ethyl acetate-acid and/or water (5:20:65:10)). Circle – the mobile phase R, cross – Q, triangle – B, square – XX, rhombus – Q/B.

and there is no longer the influence of acid content—the retention of cephalosporins for the phase Q/B, XX and B (formic acid in volume 10) is the same.

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