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COMPARATIVE STUDY OF PEPTIDE-TYPE ANTIBIOTICS IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY AND REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

ADORJAN ASZALOS* and ANGEL AQUILAR*
Division of Drug Biology, Pharmaceutical Research and Testing, Food and Drug Administration, Washington, DC 20204 (U.S.A.)

SUMMARY

Reversed-phase thin-layer chromatographic (RP-TLC) conditions were investigated for nineteen peptide-type antibiotics, with molecular weights between 102 and 25,000 and of different chemical characteristics, to find mobile phases giving R_F values between 0.05 and 0.95. For this purpose, 27 different mobile phases were employed, representing three organic modifiers, three buffers and three pH values. The suitability of these RP-TLC mobile phases for reversed-phase high-performance liquid chromatography (RP-HPLC) was then investigated. With no or only slight modification of the RP-TLC mobile phases, reasonable t_R values were obtained for the same antibiotics, using a Vydac C_{18} RP-HPLC column. It is suggested that these developed systems are applicable for other peptide antibiotics not tested in this study. However, no empirical correlations between molecular weights, R_F, t_R, theoretical plate height or plate number could be detected.

INTRODUCTION

Several investigators have explored the possibility of developing high-performance liquid chromatography (HPLC) systems based on thin-layer chromatographic (TLC) results. Golkiewicz[1] found that the relationship between TLC and HPLC values can be described by the equation

$$R_F = 1/(1 + k')$$

where k' is the capacity factor; he also found that this equation holds more reliably if sandwich-type TLC equipment is used[2]. The same observations were made by Soczewinski and Kuczmierczyk[3]. Hara[4] investigated the predictability of transferring a silica gel TLC system to HPLC for organic reaction products and intermediates, and found that the relationship between TLC and HPLC data can be described by the equation

* Graduate student, American University, Washington, DC, U.S.A.

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$$R_F \times 3 = 2/(1 + k')$$

Gilpin and Sisco[5] reported that TLC data can be used most reliably to predict HPLC data, if dodecyl hydrocarbon chains are used to derivatize the support for both purposes. This type of derivatized support was also used by Von Arx and Faupel[6] to study the relationship of TLC and HPLC data for a few classes of compounds, including steroids and penicillins, and for calcitonin. They found that within each class of compounds, as represented by three or four members of the class, a good correlation could be found in terms of chromatographic mobility. However, in their study, only a few members of each class of compounds were used and the chemical similarity was close among the members.

The basic correlation equation between TLC and HPLC, as shown above, was revised by Buglio and Venturella[7]. These authors found that k' can be predicted from R_F values by the equation

$$k' = K_{tR}\,[(1 - R_F)/R_F]$$

where $K_{tR} = (w_a/v_m)_{col}/(w_a/v_m)_{TLC}$, and w_a is the weight of the absorbent and v_m is the volume of the mobile phase in the column and the TLC plate. This relationship was applicable to adsorption but not to distribution chromatography. Recently, Rabel[8] reviewed the applicability of TLC data to HPLC and concluded that low R_F values, *i.e.*, $R_F < 0.4$, relate well to HPLC data, but higher R_F values do not.

We investigated the possibility that TLC systems in which peptide antibiotics have useful R_F values ($0.1 < R_F < 0.9$) could be used in HPLC for this type of compound. We used reversed-phase (RP) systems and 27 mobile phases, and found that RP-TLC mobile phases could be employed in RP-HPLC for peptide antibiotics, in general. However, some TLC solvent systems must be modified slightly for HPLC purposes.

EXPERIMENTAL

Nineteen peptide-type antibiotics of various molecular weights and chemical characteristics were selected for study; they were obtained from the drug standards collection of the Food and Drug Administration or from commercial sources. These antibiotics, with some of their characteristics, are listed in Table I; other characteristics can be found in the literature[9].

For the TLC studies, reversed-phase 20 × 20 cm Uniplates (Analtech, Newark, DE, U.S.A.), 250-μm thickness and containing a fluorescent detector, were used. The chambers were glass tanks (9 × 20 × 18 cm) with well-fitting covers.

Sample solutions of the antibiotics were always freshly prepared, using the most volatile solvent possible, and were spotted with graduated (1–5 μl) glass micropipettes.

Methanol (MeOH; Burdick & Jackson Labs., Muskegon, MI, U.S.A.), acetonitrile (AcCN; Burdick & Jackson) and tetrahydrofuran (THF; MC/B Manufacturing Chemists, Norwood, OH, U.S.A.) were selected as the organic modifiers of the mobile phases. For buffers, monosodium phosphate (Fisher Scientific, Pittsburgh, PA, U.S.A.) and 1-heptane sulfonic acid sodium salt (HSA; Eastman Kodak, Rochester, NY, U.S.A.) were used. All organic reagents were of spectrophotometric grade.

TABLE I

PEPTIDE-TYPE ANTIBIOTICS IN THE STUDY OF CORRELATION BETWEEN TLC AND HPLC

Antibiotic	*General character*	*Molecular weight*	*Chemical nature*	*Use*
Cycloserine	Amino acid	102	Amphoteric	Antibacterial
Hadacidin	Amino acid	119	Acidic	Antitumor
Azaserine	Amino acid	173	Amphoteric	Antitumor
Viomycin	Cyclopeptide	686	Basic	Antibacterial
Echinomycin	Cyclopeptide, aromatic	1050	Basic	Antimicrobial
Polymyxin B_1	Lipopeptide	1220	Basic	Antibacterial
Colistin S	Lipopeptide	1250	Basic	Antibacterial
Actinomycin C_2	Cyclopeptide, aromatic	1296	Neutral	Antitumor
Bacitracin A	Cyclopeptide	1470	Basic	Antibacterial
Phleomycin	Glycopeptide, aromatic	1500	Basic	Antitumor
Bleomycin S	Glycopeptide, aromatic	1550	Basic	Antitumor
Thiostrepton	Peptide, aromatic	1650	Amphoteric	Antimicrobial
Saramycetin	Peptide, aromatic	2200	Acidic	Antibacterial
Gramacidin A	Cyclopeptide	3100	Neutral	Antibacterial
Cinnamycin	Polypeptide	5000	Amphoteric	
Duramycin	Polypeptide	5000	Amphoteric	
Neocarzinostatin	Polypeptide	8750	Acidic	Antitumor
Restrictocin	Polypeptide	15,000	Amphoteric	Antitumor
Largomycin F-II	Glycoprotein	25,000	Acidic	Antitumor

Buffer solutions were prepared by weighing the exact amount for 0.01 *M* or 0.05 *M* concentrations, adding deionized water to the proper volume and adjusting the pH to the desired point. The pH values 2.0, 3.4 and 6.6 were chosen as the most appropriate for study of the migration characteristics of the peptide antibiotics.

A dark chamber provided with ultraviolet and fluorescent lamps was used to detect the migration of antibiotics when spots were not visible.

Six antibiotics (cycloserine, viomycin, polymyxin B_1, bacitracin A, duramycin and restrictocin) were selected to screen for the best systems in the initial TLC trials.

The concentration of solvent components of the mobile phase were varied between 10 and 90% to find a concentration at which all antibiotics moved with reasonable R_F values (not 0 or 1); this procedure was applied to all three organic modifiers. Twenty-seven systems were obtained from the three buffers (0.01 *M* and 0.05 *M* phosphate, 0.01 *M* HSA), each with 80% MeOH, 60% AcCN or 40% THF at pH 2.0, 3.4 and 6.6.

The samples were spotted on the TLC plate in portions, evaporating the solvent after each application. The chambers, which contained the mobile phase to the level of 0.5 cm below the spotting line of the plate, were equilibrated at room temperature.

The TLC plates were developed in the absence of sunlight until the solvent front on the plate was about three-quarters of the way up the plate. The plate was then removed from the chamber and was examined immediately for visible or fluorescence quenching and again when the plate was completely dry. Migrations were measured and R_F values were calculated on the basis of the spot identified on the plate.

For the HPLC studies, the same antibiotics were used and were dissolved in the same solvents used in the TLC studies. The liquid chromatograph was a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A, equipped with a Model 440 detector, and Model UGK valve injector. After initial trials, a Vydac RP-18 (300 × 3.6 mm I.D.) column (Altex, Berkeley, CA, U.S.A.) was selected because it gave the least amount of tailing with these antibiotics. A flow-rate of 1 ml/min was maintained throughout the experiments. The compounds were injected with a Unimetrics (Anaheim, CA, U.S.A.) graduated syringe, and the volume was varied between 1 and 10 μl according to the amount needed to obtain readable peaks. The detector was operated at 254 nm and attenuation was 0.2. A Model 660 solvent programmer from Waters Assoc. was used for screening the mobile phase composition. A Supergrator-1 (Columbia Scientific, Austin, TX, U.S.A.), a computing integrator, was used in line with the detector to record retention times.

Buffers were prepared as for TLC and stored under refrigeration when not in use; however, solvents were allowed to equilibrate to room temperature before use. The modifiers and buffers were the same as those used in the TLC studies, and 27 solvent systems were used. The mobile phase (always freshly mixed) was filtered, using a Millipore (Bedford, MA, U.S.A.) filter, and deaerated for 15 min.

With the different solvent systems, five antibiotics were first tested to investigate their mobility. If the solvent system gave a reasonable elution time for these initial five antibiotics (more than void volume and less than 50 min), the other fourteen antibiotics were also tested. For the initial trials of the solvent composition, two high-pressure pumps were used with the Waters Assoc. Model 600 solvent programmer; this arrangement made it easier to rapidly change the concentration of the isocratic solvent composition. One of the pumps delivered a solvent with a low concentration of organic modifier and the other only the organic modifier. After the best composition was found, the solvent was mixed manually for subsequent tests.

RESULTS

The observed R_F values for the peptide antibiotics in the different solvent systems are given in Table II; when tailing of an antibiotic was observed, both ends of the tailing are given.

Antibiotics having wide variations in R_F values in the different TLC solvent systems (bacitracin, thiostrepton, echinomycin, actinomycin and restrictocin) were selected for the initial HPLC studies, which were conducted by varying the composition of the organic modifier in the 0.1 *M* phosphate and 0.01 *M* HSA solvent systems to determine the optimal solvent composition. The optimal solvent composition was expected to result in reasonable t_R values ($V_0 < t_R < 50$ min) and to satisfy most nearly the equation $R_F = 1/(1 + k')$. After the above requirements were achieved, the other fourteen antibiotics were chromatographed in the selected best solvent systems. If more than one antibiotic of the nineteen migrated with the void volume or did not elute within 50 min, the concentration of the organic modifier was again changed. The final concentrations of MeOH, AcCN and THF which satisfied the requirement that all antibiotics migrate with a reasonable R_F value were 74, 52 and 40%, respectively, compared with the concentrations of 80, 60 and 40%, respectively, that had given the best mobility in TLC. The t_R values obtained with the final solvent compositions are shown in Table III.

TABLE II

R_F VALUES OF PEPTIDE-TYPE ANTIBIOTICS IN DIFFERENT TLC SOLVENT SYSTEMS*

Antibiotic	*Buffer***	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
Cycloserine	0.01 *M* HSA	MeOH	0.63	0.69	0.64
		AcCN	0.54	0.75	0.78
		THF	0.91	0.92	0.84
	0.01 *M* Pi	MeOH	0.72	0.71	0.27
		AcCN	0.20–0.69	0.00–0.73	0.00–0.68
		THF	0.87	0.88	0.86
	0.05 *M* Pi	MeOH	0.62	0.67	0.62
		AcCN	0.63	0.88	0.68
		THF	0.87	0.86	0.88
Hadacidin	0.01 *M* HSA	MeOH	0.76	0.74	0.75
		AcCN	0.55	0.50	0.44
		THF	0.95	0.94	0.80
	0.01 *M* Pi	MeOH	0.75	0.69	0.75
		AcCN	0.71	0.73	0.67
		THF	0.97	0.96	0.99
	0.05 *M* Pi	MeOH	0.79	0.78	0.71
		AcCN	0.55	0.59	0.45
		THF	0.81	0.93	0.99
Azaserine	0.01 *M* HSA	MeOH	0.70	0.76	0.70
		AcCN	0.57	0.58	0.52
		THF	0.93	0.91	0.87
	0.01 *M* Pi	MeOH	0.73	0.71	0.76
		AcCN	0.63	0.72	0.66
		THF	0.90	0.91	0.89
	0.05 *M* Pi	MeOH	0.67	0.77	0.67
		AcCN	0.62	0.61	0.68
		THF	0.90	0.93	0.95
Viomycin	0.01 *M* HSA	MeOH	0.00	0.00	0.00
		AcCN	0.10	0.09	0.02
		THF	0.18	0.00–0.16	0.00–0.65
	0.01 *M* Pi	MeOH	0.00–0.25	0.00–0.21	0.00–0.15
		AcCN	0.17	0.00–0.14	0.00–0.12
		THF	0.58	0.20–0.46	0.12–0.65
	0.05 *M* Pi	MeOH	0.00–0.20	0.00–0.08	0.00–0.09
		AcCN	0.32	0.13	0.14
		THF	0.86	0.76	0.69
Echinomycin	0.01 *M* HSA	MeOH	0.80	0.75	0.00–0.15
		AcCN	0.77	0.81	0.78
		THF	0.32	0.12–0.46	0.00–0.44
	0.01 *M* Pi	MeOH	0.73	0.48	0.76
		AcCN	0.89	0.97	0.90
		THF	0.00–0.36	0.00–0.30	0.00–0.33
	0.05 *M* Pi	MeOH	0.00–0.77	0.00–0.81	0.00–0.76
		AcCN	0.85	0.57	0.85
		THF	0.00–0.45	0.00–0.50	0.00–0.50

(*Continued on p. 88*)

TABLE II (*continued*)

Antibiotic	*Buffer***	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
Polymyxin B_1	0.01 *M* HSA	MeOH	0.15	0.00	0.00
		AcCN	0.30	0.00	0.00
		THF	0.13–0.47	0.45	0.00–0.47
	0.01 *M* Pi	MeOH	0.19	0.00–0.05	0.00–0.13
		AcCN	0.00–0.35	0.00–0.32	0.00–0.32
		THF	0.00–0.46	0.00–0.43	0.00–0.39
	0.05 *M* Pi	MeOH	0.27	0.00–0.17	0.00–0.12
		AcCN	0.38	0.07	0.00–0.40
		THF	0.55	0.24–0.65	0.50–0.75
Colistin S	0.01 *M* HSA	MeOH	0.00–0.30	0.00–0.35	0.00–0.13
		AcCN	0.25	0.10	0.00
		THF	0.62	0.61	0.59
	0.01 *M* Pi	MeOH	0.00–0.46	0.00–0.43	0.00–0.39
		AcCN	0.25–0.61	0.00–0.69	0.00–0.55
		THF	0.61	0.68	0.63
	0.05 *M* Pi	MeOH	0.21–0.45	0.00–0.56	0.00–0.30
		AcCN	0.82	0.68	0.82
		THF	0.60	0.48–0.72	0.50–0.75
Actinomycin C_2	0.01 *M* HSA	MeOH	0.63	0.62	0.61
		AcCN	0.78	0.83	0.78
		THF	0.24	0.33	0.30
	0.01 *M* Pi	MeOH	0.65	0.69	0.72
		AcCN	0.96	0.94	0.88
		THF	0.16	0.16	0.08
	0.05 *M* Pi	MeOH	0.47	0.59	0.66
		AcCN	0.73	0.78	0.58
		THF	0.00–0.23	0.20	0.06
Bacitracin A	0.01 *M* HSA	MeOH	0.00–0.40	0.00–0.50	0.00–0.43
		AcCN	0.25	0.20	0.22
		THF	0.61	0.64	0.60
	0.01 *M* Pi	MeOH	0.00–0.66	0.00–0.58	0.00–0.53
		AcCN	0.28	0.00–0.34	0.00–0.28
		THF	0.22–0.65	0.17–0.64	0.00–0.59
	0.05 *M* Pi	MeOH	0.48	0.52	0.42
		AcCN	0.45	0.86	0.36
		THF	0.65	0.57	0.58
Phleomycin	0.01 *M* HSA	MeOH	0.00–0.22	0.00–0.14	0.00–0.13
		AcCN	0.20	0.20	0.20
		THF	0.29	0.16	0.00
	0.01 *M* Pi	MeOH	0.00–0.14	0.00–0.08	0.00–0.08
		AcCN	0.09	0.05	0.04
		THF	0.50	0.16	0.21
	0.05 *M* Pi	MeOH	0.00–0.31	0.03	0.03
		AcCN	0.12	0.05	0.08
		THF	0.68	0.40	0.28
Bleomycin S	0.01 *M* HSA	MeOH	0.12	0.00–0.90	0.00–0.10
		AcCN	0.18	0.11	0.11
		THF	0.45	0.45	0.46

TABLE II (*continued*)

Antibiotic	*Buffer***	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
	0.01 *M* Pi	MeOH	0.16	0.11	0.10
		AcCN	0.28	0.16	0.13
		THF	0.62	0.31	0.34
	0.05 *M* Pi	MeOH	0.21	0.19	0.12
		AcCN	0.28	0.21	0.36
		THF	0.84	0.70	0.72
Thiostrepton	0.01 *M* HSA	MeOH	0.78	0.81	0.79
		AcCN	0.00–0.78	0.00–0.83	0.00–0.76
		THF	0.00	0.00	0.00
	0.01 *M* Pi	MeOH	0.00–0.76	0.00–0.73	0.00–0.77
		AcCN	0.89	0.90	0.82
		THF	0.00	0.00	0.00
	0.05 *M* Pi	MeOH	0.00–0.80	0.00–0.79	0.00–0.75
		AcCN	0.00–0.82	0.00–0.70	0.00–0.82
		THF	0.00	0.00	0.00
Saramycetin	0.01 *M* HSA	MeOH	0.86	0.89	0.84
		AcCN	0.73	0.81	0.75
		THF	0.82	0.82	0.85
	0.01 *M* Pi	MeOH	0.85	0.83	0.81
		AcCN	0.91	0.91	0.83
		THF	0.75	0.73	0.62
	0.05 *M* Pi	MeOH	0.89	0.89	0.84
		AcCN	0.70	0.73	0.93
		THF	0.45–0.80	0.76	0.94
Gramicidin A	0.01 *M* HSA	MeOH	0.80	0.85	0.65
		AcCN	0.00–0.79	0.00–0.75	0.00–0.75
		THF	0.00	0.10	0.00
	0.01 *M* Pi	MeOH	0.72	0.60	0.64
		AcCN	0.00–0.90	0.00–0.96	0.00–0.88
		THF	0.00	0.00	0.00
	0.05 *M* Pi	MeOH	0.27–0.73	0.33–0.81	0.52–0.85
		AcCN	0.00–0.82	0.00–0.60	0.00–0.82
		THF	0.00	0.00	0.00
Cinnamycin	0.01 *M* HSA	MeOH	0.77	0.84	0.84
		AcCN	0.00–0.77	0.00–0.73	0.00–0.72
		THF	0.00–0.60	0.00–0.65	0.00–0.68
	0.01 *M* Pi	MeOH	0.00–0.90	0.00–0.93	0.00–0.82
		AcCN	0.89	0.96	0.88
		THF	0.00–0.50	0.00–0.50	0.39
	0.05 *M* Pi	MeOH	0.00–0.78	0.00–0.83	0.00–0.73
		AcCN	0.54	0.58	0.41
		THF	0.38–0.82	0.15–0.58	0.20–0.92
Duramycin	0.01 *M* HSA	MeOH	0.72	0.76	0.71
		AcCN	0.66	0.65	0.64
		THF	0.80	0.86	0.71
	0.01 *M* Pi	MeOH	0.70	0.75	0.75
		AcCN	0.72	0.78	0.73
		THF	0.75	0.76	0.78

(*Continued on p. 90*)

TABLE II (*continued*)

Antibiotic	*Buffer***	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
	0.05 *M* Pi	MeOH	0.67	0.71	0.65
		AcCN	0.70	0.71	0.77
		THF	0.84	0.80	0.83
Neocarzinostatin	0.01 *M* HSA	MeOH	0.35	0.35	0.31
		AcCN	0.75	0.82	0.75
		THF	0.20	0.22	0.34
	0.01 *M* Pi	MeOH	0.33	0.28	0.26
		AcCN	0.63	0.60	0.43
		THF	0.10–0.53	0.06–0.51	0.04–0.36
	0.05 *M* Pi	MeOH	0.46	0.46	0.37
		AcCN	0.70	0.72	0.57
		THF	0.42	0.42	0.30
Restrictocin	0.01 *M* HSA	MeOH	0.00	0.00	0.00–0.19
		AcCN	0.00	0.00	0.05
		THF	0.22	0.00–0.14	0.00–0.20
	0.01 *M* Pi	MeOH	0.00	0.00	0.00
		AcCN	0.00	0.00	0.00
		THF	0.52	0.00–0.26	0.00–0.22
	0.05 *M* Pi	MeOH	0.00	0.00	0.00
		AcCN	0.07	0.07	0.04
		THF	0.70	0.55	0.42
Largomycin F-II	0.01 *M* HSA	MeOH	0.70	0.64	0.63
		AcCN	0.54	0.51	0.45
		THF	0.88	0.82	0.80
	0.01 *M* Pi	MeOH	0.79	0.78	0.79
		AcCN	0.65	0.66	0.56
		THF	0.90	0.86	0.82
	0.05 *M* Pi	MeOH	0.55	0.62	0.50
		AcCN	0.60	0.76	0.61
		THF	0.85	0.85	0.82

* Reversed-phase Uniplates (Analtech).
** Pi = phosphate buffer.

TABLE III

t_R VALUES OF PEPTIDE-TYPE ANTIBIOTICS IN DIFFERENT HPLC SOLVENT SYSTEMS*

Antibiotic	*Buffer*	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
Cycloserine	0.01 *M* HSA	MeOH	4.12	5.00	3.48
		AcCN	3.21	3.21	2.94
		THF	5.48	4.05	3.25
	0.01 *M* Pi	MeOH	3.08	3.32	3.21
		AcCN	2.94	2.89	2.90
		THF	3.17	3.14	3.11
	0.05 *M* Pi	AcCN	3.53	4.30	3.37

TABLE III (*continued*)

Antibiotic	*Buffer*	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
Hadacidin	0.01 *M* HSA	MeOH	4.92	4.31	3.16
		AcCN	3.40	3.71	2.58
		THF	4.86	5.13	2.78
	0.01 *M* Pi	MeOH	3.00	3.00	3.78
		AcCN	2.92	2.55	2.54
		THF	2.94	2.60	2.58
	0.05 *M* Pi	AcCN	3.51	4.45	3.08
Azaserine	0.01 *M* HSA	MeOH	4.87	4.57	3.40
		AcCN	3.32	2.86	2.80
		THF	5.02	3.88	3.02
	0.01 *M* Pi	MeOH	3.05	3.13	2.97
		AcCN	2.89	2.70	2.68
		THF	2.87	2.78	2.73
	0.05 *M* Pi	AcCN	3.32	3.56	3.03
Viomycin	0.01 *M* HSA	MeOH	4.89	5.34	6.03
		AcCN	3.18	3.64	5.99
		THF	4.26	4.97	4.17
	0.01 *M* Pi	MeOH	3.61	6.75	3.73
		AcCN	2.91	3.25	4.75
		THF	2.86	3.27	7.08
	0.05 *M* Pi	AcCN	3.02	3.53	4.01
Echinomycin	0.01 *M* HSA	MeOH	8.89	9.08	6.86
		AcCN	6.31	6.12	5.72
		THF	10.14	9.32	7.13
	0.01 *M* Pi	MeOH	5.80	6.37	6.04
		AcCN	5.63	5.53	5.56
		THF	7.27	7.96	9.03
	0.05 *M* Pi	AcCN	5.32	6.84	6.30
Polymyxin B_1	0.01 *M* HSA	MeOH	9.34	12.57	9.42
		AcCN	3.36	4.48	4.39
		THF	4.78	4.97	3.95
	0.01 *M* Pi	MeOH	4.39	5.33	8.94
		AcCN	3.05	3.64	3.21
		THF	3.25	3.83	3.54
	0.05 *M* Pi	AcCN	3.34	3.50	3.10
Colistin S	0.01 *M* HSA	MeOH	4.69	4.70	3.75
		AcCN	3.02	3.60	3.85
		THF	4.30	4.95	3.88
	0.01 *M* Pi	MeOH	3.21	3.18	3.03
		AcCN	2.78	2.94	2.74
		THF	3.09	3.06	2.98
	0.05 *M* Pi	AcCN	3.26	3.27	3.47
Actinomycin C_2	0.01 *M* HSA	MeOH	15.64	15.61	13.24
		AcCN	18.26	17.45	17.21
		THF	19.23	14.19	13.10
	0.01 *M* Pi	MeOH	10.43	11.96	11.35
		AcCN	16.33	16.33	16.14
		THF	12.22	14.51	18.01
	0.05 *M* Pi	AcCN	17.29	18.44	18.54

(*Continued on p. 92*)

TABLE III (*continued*)

Antibiotic	*Buffer*	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
Bacitracin A	0.01 *M* HSA	MeOH	6.68	7.56	6.11
		AcCN	3.77	5.42	3.74
		THF	7.37	9,61	5.52
	0.01 *M* Pi	MeOH	4.45	5.11	3.42
		AcCN	3.64	3.88	2.76
		THF	3.48	4.46	4.17
	0.05 *M* Pi	AcCN	3.26	3.66	3.02
Phleomycin	0.01 *M* HSA	MeOH	5.48	5.44	4.32
		AcCN	3.13	4.32	3.02
		THF	4.52	6.34	3.34
	0.01 *M* Pi	MeOH	3.56	3.78	3.32
		AcCN	3.08	3.59	2.84
		THF	2.81	3.29	2.86
	0.05 *M* Pi	AcCN	3.05	3.27	2.92
Bleomycin S	0.01 *M* HSA	MeOH	5.26	5.48	5.11
		AcCN	3.06	3.80	3.48
		THF	4.63	5.47	3.77
	0.01 *M* Pi	MeOH	3.46	3.53	4.10
		AcCN	2.97	3.24	3.21
		THF	2.84	3.18	3.10
	0.05 *M* Pi	AcCN	3.25	3.46	3.02
Thiostrepton	0.01 *M* HSA	MeOH	10.38	9.04	9.64
		AcCN	4.82	6.28	5.96
		THF	15.93	16.12	12.20
	0.01 *M* Pi	MeOH	7.13	8.39	7.69
		AcCN	5.05	5.69	5.67
		THF	11.26	15.24	11.85
	0.05 *M* Pi	AcCN	6.30	6.65	6.45
Saramycetin	0.01 *M* HSA	MeOH	5.13	3.16	3.02
		AcCN	3.13	2.86	2.33
		THF	5.26	4.01	2.54
	0.01 *M* Pi	MeOH	3.05	2.76	2.81
		AcCN	2.84	2.60	2.25
		THF	4.59	3.78	3.01
	0.05 *M* Pi	AcCN	3.32	3.26	2.68
Gramicidin A	0.01 *M* HSA	MeOH	–	–	34.46
		AcCN	–	–	–
		THF	–	–	–
	0.01 *M* Pi	MeOH	25.69	31.71	26.71
		AcCN	–	–	–
		THF	–	–	–
	0.05 *M* Pi	AcCN	–	–	–
Cinnamycin	0.01 *M* HSA	MeOH	5.27	4.20	3.77
		AcCN	3.83	3.47	2.78
		THF	5.00	4.89	3.50
	0.01 *M* Pi	MeOH	3.29	3.08	3.00

TABLE III (*continued*)

Antibiotic	*Buffer*	*Modifier*	*Buffer pH*		
			2.0	*3.4*	*6.6*
		AcCN	3.33	3.23	3.10
		THF	3.22	3.29	3.24
	0.05 *M* Pi	AcCN	4.00	3.83	3.51
Duramycin	0.01 *M* HSA	MeOH	5.62	4.09	3.75
		AcCN	3.34	3.08	2.84
		THF	4.71	4.07	3.21
	0.01 *M* Pi	MeOH	3.28	3.05	3.00
		AcCN	3.20	2.78	2.77
		THF	3.67	3.81	3.16
	0.05 *M* Pi	AcCN	3.61	3.53	3.10
Neocarzinostatin	0.01 *M* HSA	MeOH	5.67	3.91	2.65
		AcCN	3.40	3.21	2.20
		THF	4.62	3.80	2.46
	0.01 *M* Pi	MeOH	3.29	2.60	2.17
		AcCN	2.92	2.52	2.14
		THF	3.26	2.49	2.20
	0.05 *M* Pi	AcCN	3.29	2.97	2.54
Restrictocin	0.01 *M* HSA	MeOH	5.33	4.62	3.74
		AcCN	3.24	3.02	2.84
		THF	4.04	4.30	2.81
	0.01 *M* Pi	MeOH	4.78	6.18	2.68
		AcCN	3.05	4.40	2.46
		THF	2.86	3.36	2.54
	0.05 *M* Pi	AcCN	3.54	3.50	2.97
Largomycin F-II	0.01 *M* HSA	MeOH	5.49	4.30	3.34
		AcCN	3.56	3.49	2.61
		THF	5.03	5.34	2.20
	0.01 *M* Pi	MeOH	3.32	5.84	2.72
		AcCN	3.30	2.84	2.02
		THF	3.28	2.98	2.58
	0.05 *M* Pi	AcCN	4.21	3.50	2.77

* Vydac RP-18 column.

TABLE IV

PLATE HEIGHT NUMBERS (*H*) AND THEORETICAL PLATE NUMBERS (*N*) FOR PEPTIDE-TYPE ANTIBIOTICS

Vydac RP-18 column; mobile phase, 0.1 *M* phosphate buffer, pH 2.0, with organic modifier.

Antibiotic	*Methanol (74%)*			*Acetonitrile (52%)*			*Tetrahydrofuran (40%)*		
	H (mm)	*N*	t_R	*H (mm)*	*N*	t_R	*H (mm)*	*N*	t_R
Azaserine	0.18	1343	3.05	0.15	1484	2.89	1.60	1600	2.87
Echinomycin	0.27	292	5.80	0.76	323	5.63	0.11	2367	7.27
Actinomycin	0.32	771	10.43	0.27	846	16.30	0.30	1532	12.22
Thiostrepton	0.24	1024	7.13	0.15	1600	5.05	0.17	1393	11.09
Bacitracin A	0.51	489	4.45	0.36	660	3.64	0.46	513	3.48
Restrictocin	2.90	83	4.78	1.14	217	3.05	1.20	196	2.86

From the data shown in Tables II and III, peak-width measurements and column length, several calculations were made. The theoretical plate numbers, N, for the antibiotics were calculated by using the equation

$$N = 16\,(t_R/t_w)^2$$

where t_w = band-width and N and t_R were as defined above[10]. Results of these calculations for the antibiotics selected for the initial HPLC studies and for azaserine are shown in Table IV.

The theoretical plate height numbers, H, for the antibiotics were calculated by using the equation

$$H = L/16\,(w_t/t_R)^2$$

where L = column length and w_t = peak width at the baseline[11].

To correlate TLC and HPLC data, the equation

$$R_F = 1/(1 + k')$$

was used, where $k' = (t_R - t_0)/t_0$ and t_0 = void volume[12]. Results of the calculations for six antibiotics are shown in Table V.

DISCUSSION

We have investigated the possibility that RP-TLC solvent systems could be used to chromatograph peptide-type antibiotics in RP-HPLC. The nineteen peptide-type antibiotics investigated varied in molecular weight from 102 to 25,000 and were of different chemical natures. Some are used clinically in chemotherapy (bacitracin, cycloserine, polymyxin G, colistin S, actinomycin, bleomycin, gramicidin A and neocorcinostatin) and some are used in biochemical investigations (echinomycin, thiostrepton, largomycin F-II and restrictocin). Besides providing TLC and HPLC systems for these antibiotics, the application has promise for other peptide antibiotics not included in this study.

Other investigators have dealt with the usefulness of TLC solvent systems in HPLC[1–8]. In these investigations, classes of compounds having closely similar physicochemical natures were studied, and several empirical relationships were detected. We have found that solvent systems of RP-TLC with the Analtech C_{18} stationary phase, *i.e.*, 0.01 *M* and 0.05 *M* phosphate and 0.01 *M* HSA buffers with MeOH, AcCN and THF organic modifiers at pH values of 2.0, 3.4 and 6.6 can be used with little or no modification in RP-HPLC when a Vydac RP-18 column is used. The Vydac column gave the least amount of tailing for these peptide-type antibiotics. The solvent systems considered to be useful were those having a mobility of $V_0 < t_R \leq 50$ min. Changing the concentration of MeOH from 80 to 74% and the concentration of AcCN from 60 to 52% in all buffers and at all pH values provided acceptable mobility for all antibiotics we tested in RP-HPLC. Acceptable RP-HPLC results were achieved without a change in THF concentration.

We concluded that these antibiotics, in spite of their wide variation in molecular weight and physicochemical nature, behave very similarly in RP-HPLC. Since

the antibiotics we investigated included all types of possible peptide antibiotics, these developed RP-TLC and RP-HPLC conditions should provide potentially useful chromatographic systems for other antibiotics of the same class.

Previous investigators who related TLC data to HPLC used closely similar compounds in their studies[1–7], which have provided empirical formulae between R_F and t_R values.

However, in reviewing the field, Rabel[8] concluded that these relationships hold only if R_F values are below 0.4. In addition, Golkiewicz[2] showed that correlations exist between TLC and HPLC only if a sandwich-type TLC chamber is used.

We were interested in exploring the relationship of R_F and t_R values as well as the relationship of H and N to the molecular weight and chemical nature of these peptide-type antibiotics.

Comparison of the molecular weights (Table I) with the R_F values (Table II) and t_R values (Table III) indicated that no correlation equation can be written for these values. For example, the smaller molecular weight peptides cycloserine and hadacidin do not travel faster in any of the TLC systems than larger peptide antibiotics such as saramycetin or duramycin. A similar generalization can be made between the t_R values, molecular weights and chemical nature of the antibiotics. The relationship of R_F and t_R values was compared, using the equation $R_F = 1/(1 + k')$. Results for the antibiotics selected for the initial HPLC studies and for azaserine, a low-molecular-weight antibiotic, obtained with 0.01 M phosphate buffer with the three organic modifiers at pH 2.0, are given in Table V for illustrative purposes. As can be seen, the above equation does not hold and this conclusion is valid for the other antibiotics tested. In addition, no other type of empirical relationship could be detected between these values. It should again be emphasized that the selected solvent systems provided useful chromatographic (TLC and HPLC) conditions for these peptide antibiotics. However, within these limits no close relationships exist among molecular weights, R_F and t_R values. The reason for this lack of correlation is not

TABLE V

RESULTS OF CALCULATION FOR PEPTIDE-TYPE ANTIBIOTICS WITH THE TLC–HPLC CORRELATION EQUATION

Vydac RP-18 column; mobile phase, 0.01 M phosphate buffer, pH 2.0, with organic modifier.

Antibiotic	*Methanol**		*Acetonitrile***		*Tetrahydrofuran****	
	R_F *(found)*	R_F *(calculated)*	R_F *(found)*	R_F *(calculated)*	R_F *(found)*	R_F *(calculated)*
Azaserine	0.72	0.48	0.69	0.51	0.87	0.47
Echinomycin	0.73	0.43	0.63	0.52	0.9	0.52
Actinomycin	0.73	0.25	0.89	0.26	0.36	0.21
Thiostrepton	0.76	0.21	0.89	0.30	0.00	0.13
Bacitracin A	0.66	0.34	0.28	0.41	0.65	0.34
Restrictocin	0.00	0.28	0.00	0.43	0.52	0.52

* 80% for R_F and 74% for calculated $R_F = 1/(1 + k')$.
** 60% for R_F and 52% for calculated R_F.
*** 40% for both R_F and calculated R_F.

known, but most likely the hydrodynamic shapes of the peptide antibiotics and their distribution characteristics in the phases are similar and play an important role in their migration characteristics.

We also investigated whether any relationship existed between the theoretical plate number, N, the chemical nature of the antibiotics and their t_R values. Again, no trend of any sort could be detected between these values (Table IV); for example, echinomycin, actinomycin, thiostrepton and bacitracin, which have about the same molecular weights (1050–1650), showed considerable variation in N. The same conclusion can also be made for the other antibiotics tested under these chromatographic conditions. We reached a similar conclusion after comparing the theoretical plate height, H, with t_R values and the chemical nature of the antibiotics (Table IV).

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