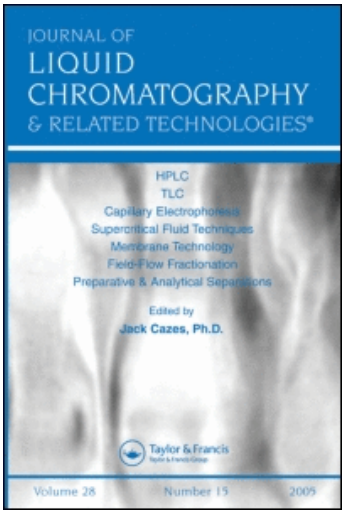

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Thin Layer Chromatographic Systems for the Classification and Identification of Antibiotics

Adorjan Aszalos[a]; Haleem J. Issaq[b]
[a] Food and Drug Administration, Washington, D.C. [b] Chemical Carcinogenesis Program Frederick Cancer Research Center, Frederick, Maryland

To cite this Article Aszalos, Adorjan and Issaq, Haleem J.(1980) 'Thin Layer Chromatographic Systems for the Classification and Identification of Antibiotics', Journal of Liquid Chromatography & Related Technologies, 3: 6, 867 — 883

To link to this Article: DOI: 10.1080/01483918008060198

URL: http://dx.doi.org/10.1080/01483918008060198

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(6), 867-883 (1980)

THIN LAYER CHROMATOGRAPHIC SYSTEMS FOR THE CLASSIFICATION AND IDENTIFICATION OF ANTIBIOTICS

Adorjan Aszalos*
Food and Drug Administration
HFD-436
Washington, D.C. 20204

and

Haleem J. Issaq
Chemical Carcinogenesis Program
Frederick Cancer Research Center
Frederick, Maryland 21701

INTRODUCTION

Several comparative chromatographic systems described in the literature may be useful for facilities dealing with a relatively large number of different antibiotics, such as clinical laboratories engaged in the

* Author to whom correspondence should be addressed.

identification of antibiotics in human serum; research laboratories screening for new antibiotics; and regulatory agencies testing drug preparations. Ishida et al. (1) introduced the "summarized paper chromatogram" which is obtained when R_f values computed from several solvent systems are represented graphically. Betina (2) classified 62 antibiotics on the basis of graphical representation using paper chromatographic techniques. Later Betina and Nemec (3) used paper chromatography (PC) with buffered solutions for the classification of antibiotics. Snell et al. (4) used paper chromatographic classification of antibiotics in crude preparations.

Thin layer chromatography (TLC), however, has several advantages over paper chromatography. It takes less time than PC, the spots can be quantitated in situ on the plate (5) and it is easier to automatically elute the spots from the plate for ancillary techniques such as infared, mass spectrometry, uv-visible and nuclear magnetic resonance, for structural identification (7).

Issaq et al. (8) used TLC for the classification of 150 antibiotics exhibiting antitumor properties. Aszalos et al. (9,10) used instant TLC for the classification of 91 antibiotics. Kreuzig (11) discussed the use of high performance TLC in the antibiotic field. This review discusses several of the TLC classification and identification systems used in the antibiotic field.

METHODS

Thin layer chromatography: One of the earliest systems used for the identification of antibiotics by TLC was described by Ito et al. (12). They used cellulose as the adsorbent and propanol:pyridine:acetic acid:water (15:10:3:12) as the developing solvent for the separation of 19 water-soluble antibiotics. Ninhydrine or oxidized nitroprusside were

used to visualize the compounds. Table I shows the R_f values of the antibiotics studied with this system. Forty-two antibiotics, available commercially in France in pharmaceutical preparations, were classified into groups by Schmitt and Mathis (13) using TLC solvent systems horizontally (simultaneously). They concluded that chemically related antibiotics fell into the same group and, therefore, TLC grouping can provide information about the general chemical characteristics of an antibiotic. However, this conclusion was not substantiated by studies made by Aszalos' group as will be discussed later. Antibiotics were applied to Kieselghur plates in 1-3 µl quantities. After development, the antibiotics were identified by their R_f values and by the characteristic colors visible after the plates had been sprayed with Mathis-Schmitt solution (14). The results of this study are presented in Tables II and III.

Table I. TLC results (R_f) of basic water-soluble antibiotics on cellulose 300 (MN) (12).

Antibiotic	R_f x 100
Glebomycin	41
Streptomycin	44
Dihydrostreptomycin	44
Hydroxystreptomycin	32
Netropsin	51
Amidinomycin	53
Gentamycin	35
Streptothricin	26
Viomycin	21
Kanaymcin A	17
Kanamycin B	15
Kanamycin C	23
Paromomycin	15
Zygomycin	15
Catenulin	15
Neomycin	10
Fradomycin	10

Table II. TLC results of antibiotics on Kieselghur G (Merck) in four solvent systems (13).

Antibiotic	R_f x 100			
	I[a]	II[a]	III[a]	IV[a]
Triacetyloleandomycin	14	98	51	0
Griseofluvin	98	98	78	0
Virgimycin	58	79	79	10
Pristinamycin	33	35	-	-
	57	82	80	0
	92	87	-	51
Novobiocin (Na)	38	61	92	0
Dihydronovoiocin (Na)	12	59	90	0
Dihydronovobiocin	60	66	95	0
Fusidic acid (Na)	79	67	98	0
Rifamycin	99	17	88	0
Penicillin G (Na)	95	30	91	48
Cephalothin (Na)	22	38	83	44
Cephaloridine	20	25	26	12
Chloramphenicol	66	76	87	66
Propiocine	0	100	52	0
Erythromycin	0	50	52	0
Spiramycin	0	55	35	10
			56	
Oleandomycin (PO_4)	0	15	18	10
Lincomycin (HCl)	0	80	42	29
Kitasamycin (tartrate)	0	85	77	0
Cycloserine	0	33	86	87
Hydroxymethylgramicidin	0	67	97	0
Tyrothricin	0	58	66	0
			100	
Bacitracin	0	0	54	0
Pimaricin	0	0	59	0
Nystatin	0	0	55	0
Trichomycin	0	0	57	0
Tetracycline	0	0	47	23
Methylenecycline (HCl)	0	0	53	33
Oxytetracycline	0	0	45	40
Demethylchlortetrachycline (HCl)	0	0	52	26
Chlortetracycline (HCl	0	0	49	25
Rolitetracycline	0	0	47	23
Colimycin (SO_4)	0	0	33	0
Polymixin B (SO_4)	0	0	18	10
Streptomycin (SO_4)	0	0	8	90
Dihydrostreptomycin (SO_4)	0	0	6	91
Neomycin (SO_4)	0	0	0	95
Kanamycin (SO_4)	0	0	0	83
Paromomycin (SO_4)	0	0	0	91
Framycetin (SO_4)	0	0	0	91
Gentamicin (SO_4)	0	0	0	46
Viomycin (SO_4)	0	0	0	83

[a] Solvent I: chloroform:methanol:acetic acid (90:8:2)
Solvent II: chloroform:methanol:water (80:20:25)
Solvent III: butanol:acetic acid:water (50:25:25); before use of this solvent system, chromatoplates were impregnated with pH 3 buffer (potassium phosphate)
Solvent IV: water:sodium citrate:citric acid (100:20:5).

Table III. TLC results of antibiotics on Kieselghur G (Merck) in six solvent systems (13).

Antibiotic	R_f x 100					
	Ether	Ethyl Acetate	Acetone	Methanol	Ethanol	Water
Triacetyloleandomycin	1	15	90	75	50	0
Griseofluvin	30	70	100	70	70	0
Virgimycin	8	30	90	80	90	10
Pristinamycin	15	30	80	80	70	20
Novobiocin (Na)	5	20	95	85	90	0
Dihydronovoiocin (Na)	5	20	95	85	90	0
Dihydronovobiocin	20	40	95	90	95	45
Fusidic acid (Na)	10	30	80	80	70	0
Rifamycin	0	3	100	85	80	65
Penicillin G (Na)	0	0	0	85	50	80
Cephalothin (Na)	0	0	0	80	65	80
Cephaloridine	0	0	0	0	10	20
Chloramphenicol	35	60	100	100	100	90
Propiocine	10	30	75	75	65	20
Erythromycin	0	0	10	40	15	100
Spiramycin	5	15	55	70	90	10
Oleandomycin (PO_4)	0	0	3	30	20	10
Lincomycin (HCl)	0	0	25	75	80	15
Kitasamycin (tartrate)	3	15	95	90	100	60
Cycloserine	45	70	80	90	95	90
Hydroxymethylgramicidin	0	0	20	100	100	0
Tyrothricin	0	0	0	100	100	0
Bacitracin	0	0	0	30	2	0
Pimaricin	0	0	0	0	50	40
Nystatin	0	0	0	100	0	0
Trichomycin	0	0	0	20	65	0
Tetracycline	0	0	0	100	10	0
Methylenecycline (HCl)	0	0	0	100	75	10
Oxytetracycline	0	0	0	20	50	10
Demethylchlortetrachycline (HCl)	0	0	0	100	25	10
Chlortetracycline (HCl	0	0	0	100	25	10
Rolitetracycline	0	0	0	20	30	10
Colimycin (SO_4)	0	0	0	0	0	0
Polymixin B (SO_4)	0	0	0	0	0	0
Streptomycin (SO_4)	0	0	0	0	0	40
Dihydrostreptomycin (SO_4)	0	0	0	0	0	40
Neomycin (SO_4)	0	0	0	0	0	50
Kanamycin (SO_4)	0	0	0	0	0	70
Paromomycin (SO_4)	0	0	0	0	0	65
Framycetin (SO_4)	0	0	0	0	0	40
Gentamicin (SO_4)	0	0	0	0	0	25
Viomycin (SO_4)	0	0	0	0	0	60

The simultaneous use of different TLC solvent systems does not lend itself for the identification and classification of a large number of compounds. Therefore, when a method was developed for the identification of 150 antitumor antibiotics, present in crude fermentation mixtures or in pharmaceutical preparations, vertical (sequential) as well as horizontal solvent systems were used (8). The method was designed for the fast evaluation of crude antibiotic preparations obtained from fermentation media or mixed in pharmaceutical preparations. Using this chromatographic system, R_f values can be regarded as indications rather than the basis of the classification. R_f values may be influenced by the presence of impurities. Therefore, mobility or its absence (Rf 0.0-0.05) in any solvent system can only classify antibiotics into groups and subgroups. On this basis, the 150 antitumor antibiotics were grouped into 5 main and 19 subgroups. The solvent systems used are shown in Figure 1. The antibiotic groups and subgroups together with R_f values obtained in the individual solvent systems are presented in Tables IV-VIII. In another study Aszalos et al. (10) used instant TLC to classify 91 antibiotics into five groups.

Bioautography: Crude preparations or pharmaceutical mixtures contain many biologically inactive impurities that yield multiple spots on the TLC plates. The antibiotics are located on the TLC plates by bioautography, a technique which utilizes agar plates seeded with sensitive microorganisms or mammalian cells. The biologically active material on the TLC plate is located by placing the plate on the seeded agar and incubation to find out where cell growth is inhibited. The inhibited spots are regarded as R_f value on the TLC plate.

Typically, bioautographic agar for microorganism consists of a base layer covered with a second layer seeded with the microorganism. The

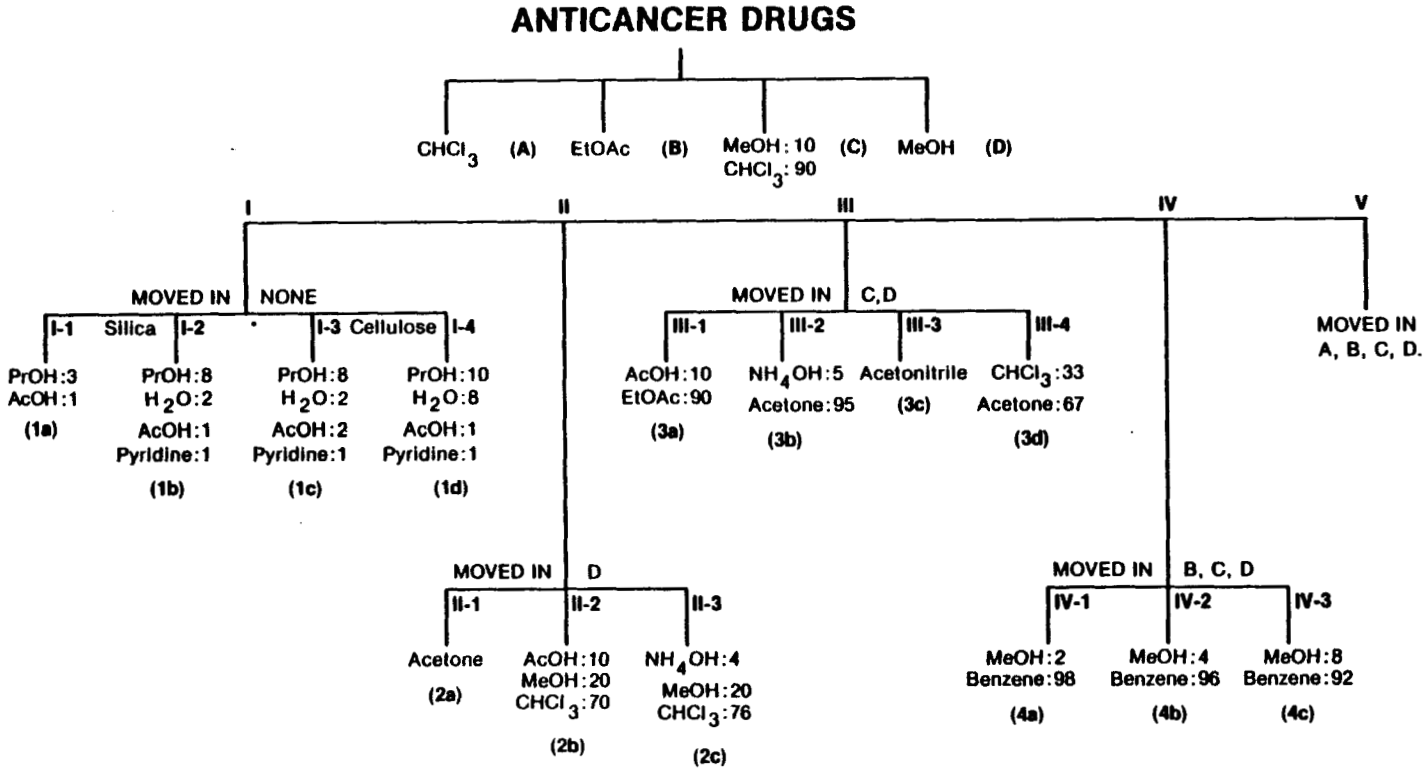

Figure 1. Solvent system used in classification of 150 antitumor antibiotics by TLC methods (8). Silica gel plates were used except for I-3 and I-4. (By permission of Elsevier Sci. Publ. Co., Amsterdam)

Table IV. Subgroups of antitumor antibiotics which moved with none of the main solvents (main group I) (8). S = streaking from R_f to R_f. R_f values expressed as R_f x 100.

		Silica Gel	Cellulose		Silica Gel	
		Solvent System				
Subgroup	Name or NSC #	A,B,C,D	1a	1b	1c	1d
I-1	Asparaginase	0	0	0	0	0
	Mitogillin	0	0	0	0	0
	Mitosper	0	0	0	0	0
	Restrictocin	0	0	0	0	0
	75603	0	0	0	0	0
I-2	Actinogan	0	0	0	0	0-20s
	Bleomycin A1	0	0	0	0	0-35s
	Bleomycin A2	0	0	0	0	85
	Carzinostatin	0	0	0	0	0,82
	Flammulin	0	0	0	0	0-30s
	Gougerotin	0	0	0	42	0
	Macromomycin	0	0	0	0	75s,801
	Neocarzinostatin	0	0	0	0	80
	Peptinogan	0	0	0	0	10
	Roseolic Acid	0	0	0	0	86
	α-Sarcin	0	0	0	0	0-35s
	Trienine	0	0	0	0	73
	116328	0	0	0	0	68,90
I-3	Alanosin, Monosodium Salt	0	0	+	0	40
	Bluensomycin Sulfate	0	0	0	50,30	38
	Kasugamycin	0	0	+	0	60
	Phleomycin	0	0	+	0	25s,82
	Sancyclin	0	0	87	0	16
	Septacidin	0	0	0	85	30
	Sistomycosin	0	0	0	80	77
	26697	0	0	0	0s	s
I-4	Actinorubin	0	0	+	10s	0-41s,80
	3-amino-2,36-L Hexopyronase HCl	0	0	+	60	82
	Adriamycin	0	0	75	73	87
	Cinnamycin	0	0	+	+	85
	Daunomycin	0	0	79	77	90
	Duramycin	0	0	+	-	80,85,45
	Hadacidin	0	0s	+	40	64
	Iyomycin Complex	0	0	+	21s	100
	Nisin	0	0s	20	0-30s	0-50s
	Spectinomycin	0	0	+	23	60
	Zorbamycin	0	0	0-10s	25,5, 82	99 0-64s
	72942	0	0	+	18	-50s
I-5	PA 147	0	10	+	+	86
	Candicidin	0	60s	10	80	0-75s
	Copiamycin, Acetyl	0	94	+	+	88
	Hedamycin	0	0-1s	+	+	50,82,0
	Nucleoside Fraction of Septacidin	0	21-50s	+	0	63,87
	Oosporin	0	45-50s	+	0	45-50s
	Stendomycin Salicylate	0	83	+	+	70

Table V. Subgroups of antitumor antibiotics which moved with methanol only (main group II) (8). See legend Table IV.

Subgroup	Name or NSC #	Silica Gel Solvent System D	2a	2b	2c
II-1	Azacolution Complex	0,40	0	0	0
	Azalomycin F-complex	14,50	0	0	0
	Azotomycin	86	0	0	0
	Cytovirin	0,14	0	0	0
	Duazomycin	s	0	0,s	0,s
	Indole-3-Carboxaldehyde	75	0	0	0
	Statolon	0-33s	0	0	0
II-2	5-Azacytidine	59	0	29	0
	Azaserine	s	0	10	0
	DON	s	0	10	0
	Formycin B	76	0	31	0
	3H-Indole	13	0	0	92
	Pyrazomycin	78	0	25	0
	Rubiflavin	10s	0	0	58,98
	Sarkomycin, Sodium Salt	68	0	9,73	0
II-3	c AMP	74	28	0	39
	Formycin A	67	0	29	12
	Palmitoyl-citidine	94	0	77	33
	Sangivamycin	71	0	26	21
	Thiosangivamycin	75	0	42	18
II-4	Acrylamide	86	65,86	52	10
	Actinobolin	36	0-25s	11,13	10,18
	Adenosine	35	30	21	61
	L-Lyxo-Hexopyranoside	18	0,22	50	58
	Mitocromin	79	10	100,95 82,72	100,71 41,40
	Rufochromomycin	82	0-9s,10	68s	0,6,15
	Steptonigrin	86	21	15s,63	21

(By permission of Elsevier Sci. Publ. Co., Amsterdam)

composition of the second layer varies according to the nutritional requirement of the microorganism employed. The dried TLC plate is then placed on sterilized filter paper resting on the seeded agar for approximately 3 hr to allow elution of the bioactive material into the agar. After removal of the TLC plate and the filter paper and incubation for optimal time and temperature for microbial growth, the zone of inhibition

Table VI. Subgroups of antitumor antibiotics which moved with methanol and 10% methanol in chloroform solvents (main group III) (8). See legend Table IV.

Subgroup	Name or NSC #	Silica Gel Solvent System C	D	3a	3b	3c	3d
III-1	Iyomycin B_1	0-10	0-10	0	0	0	0
	Mithramycin	16	92	0	0	0	0
	Mithramycin-Mg	18	92	0	0	0	0
	Sparsomycin	16	67	0	0	0	0
III-2	Aureolic Acid	16	92	0	0	96	0
	Amicetin	60	80	0	14-45	0	0
	Anisomycin	15	40	0	13	0	0
	Logosin	17	90	0	13	0	0
III-3	Azastreptonigrin	34	91	23	0	0	35,0
	Nebularin	18	71	0	29	24	0
	Puromycin	15	40	0	0-12	0	81
	Steptonigrin methyl ester	11	88	0-35	0	0-15	0
	Vinblastine, sulfate, hydrate	72,41	55	0	92	0	21
III-4	Ascomycin	90,0	70	96,0	96,0	34,0	
	Chartreusin-2-hydrate	40	70	50,26	0	0-25	18-37
	Cordycepin	13	60	17	45	21	0
	Fusidic Acid	0	24	85	0	96	36
	Steptozotocin	20,0	80	45,0	34,23	0	30,20
III-5	Antibiotic M5-18903	60,50	88	-	96	98	25
	Antibiotic 1037	13	70	32	45	75	16
	Olivomycin	50	90	-	98	98	41
	Chromomycin A2	47	95	45	94	98	48
	Mitomycin C	18	78	18	74	75	23
	Olivomycin A	50	95	26-50	93	98	40
	Pactamycin	44	70	12	93	93	78
	Rifamycin SV	62,10	92	90,57	93	98	56
	Steptolydigin	0-45	85,77	0-20	55	0-14	28
	Steptozotocin HCl	62,25,0	80	19	21	0-16	11

(By permission of Elsevier Sci. Publ. Co., Amsterdam)

is located and expressed as R_f value. Details of this procedure can be found in the literature (8,9). Betina (15) wrote a critical review of the application of bioautography as a special detection method in paper and TLC studies on antibiotics.

Table VII. Subgroups of antitumor antibiotics which moved with methanol, 10% methanol in chloroform, and ethylacetate solvents (main group IV) (8). See legend Table IV.

Subgroup	Name or NSC #	Silica Gel Solvent System					
		B	C	D	4a	4b	4c
IV-1	Antibiotic E73	50	76	81	20	40	-
	Cinerubin	s	94	s	30	46	92
	Cycloheximide	73,50	-	80	-	62,37	86,662
	Giotoxin	75	90,80,70	87	10	30	40
	Griseofulvin	52	92	82	10	16	50
	Verrucarin A	80,75	98,95	90	10	14	50
	Nonactin	86	30	85	40	40	50
	Prodigiosin	46	71,33	50	12	17	35,15
	Steptorubin	63	90	71	10s	31,17s	50,36s
	T-2 Toxin	76	79	88	10	10	-
	Tuberin	42	43	83	12	18	29
IV-2	Famagillin	38	0-43	89	0	10	-
	Fusarubin	68	80	82	0	17	43
	Mycorhodin	38	95	89	0	10	-
	Oligomycin	86	90	89	0	10	15
	Streptovitacin A	15	40	80	0	11	30
	Streptovitacin B	48	78	92	0	10	14
	Streptovitacin C	48	71	92	0	10	18
	Streptovitacin D	69	88	92	0	10	33
	Streptovitacin G	52	76	92	0	10	14
IV-3	Antibiotic B-14798-X	42	43	83	0	0	15
	Actinomycin C2	24	50,40,30	86	0	0	20
	Actinomycin C3	25	55,45,35	86	0	0	18
	Actinomycin D	24	50,40,30	86	0	0	20
	Carbomycin	30	72,58,54	92	0	0	16
	Chloramphenicol	42	43	83	0	0	15
	Cyanein	40	33	88	0	0	10
	Enteromycin	13	17,04	87	0	0	10
	Illudin	56	44	88	0	0	13
	Mikamycin	75, 25,17	82,70 0-50	82,40	0	0	22
	Porfiromycin	12	32	78	0	0	14
	Rifamide	14,0	62,25,0	90	0	0	10
	Toyocamycin	10	23	80	0	0	50
	Viridogrisein	0-15	80	85	0	0	21
IV-4	Anguidine	50	14	88	0	0	0
	Gelbecidine	0-16	81	91	0	0	0
	Narangomycin	18	65	78	0	0	0
	Rubradirin	63,0	98,75,0	87,0	0	0	s
	Ryanodine	50	14	88	0	0	0
	Streptovaricin A	24	43	95	0	0	0
	Kundrymycin	0-11	18-27	82	0	0	0

Table VIII. Antitumor antibiotics which moved with all main solvents (main group V) (8). See legend Table IV.

Name or NSC #	Silica Gel Solvent System A	B	C	D
L-Alanosine	45	40	80	75
Coumermycin	0-10	10-18	0-23	100
Cyclamycin Complex	0-14	0-12 20,60	30,90	60,80
Kanchanomycin	0-20	0	0,60,80	0-24
11254	96	96	95	95
58987	28	41	77	93
102810	10,20	91	93	91
Bostrycoidin	10	0-60	80	0-36
108408	60	88	100	85
135015	0-60	40	75	75

S = streaking for Rf to Rf.
Rf values are expressed as Rf x 100.
(By permission of Elsevier Sci. Publ. Co., Amsterdam)

For the bioautography of certain antibiotics, mammalian cells are preferable to microorganisms. Among other mammalian cells, KB cells are used successfully in semisolid agar plates. The roller bottle produced cells are spun down and resuspended to a final concentration of 10^6 cells per ml medium at 37°C and are immediately dispersed into the bioautographic dishes. (For KB cells medium MEMgo plus 15% calf serum is recommended.) After cooling to room temperature, the TLC plates are treated as with microorganisms. The plates are kept at 37°C overnight, in humidified CO_2 incubators. Detection of inhibition zones is best done by flooding the agar surface with 2,6-dichlorophenol indophenol for 5-10 min. After pouring off the dye, the bioautograph is placed in the incubator for 40-60 min. The dye is reduced by viable cells causing a color change from blue to white. Blue spots, the area of dead cells, are regarded as R_f values on the TLC plates.

DISCUSSION

Using the above techniques (i.e. TLC combined with bioautography) the results given in Tables IV-VIII were obtained with reference samples. These results were later used for the identification of antitumor antibiotics in fermentations media (10). It was found that the antibiotics in mixtures, fermentation media or crude preparations may not give the same R_f value (migration) as those of pure antibiotics. However, the main and subclasses for all antibiotics remained the same. If one pure antibiotic did not move in one particular solvent system, the same occurred with the antibiotic in the impure preparation, mainly because of the design of the solvent systems. It is possible, therefore, to eliminate the use of standard samples of antibiotics, usually used in parallel identification processes, after the system has been standardized in a particular laboratory. In addition to classifying an antibiotic, the system may help in the indentification of antibiotics in pharmaceutical preparations. Also, preliminary indications may be obtained as to whether an antibiotic in an unknown mixture is a novel one. For example, antibiotics FCRC-53 (16), FCRC-48 (17), and FCRC-57 (18) were detected in the author's laboratories using this technique. Furthermore, the classification system can be stored in a computer and used for the evaluation of an antibiotic in a fermentation media (19).

Examination of the data in Tables IV-VII reveals that the solvent systems employed did not place all antibiotics with close chemical relationship into one subgroup as was suggested by Schmitt and Mathias (13). Only trends can be detected and mostly on the main group levels. For example, most nucleoside type antibiotics fell in the main group II; however, the quinone type antibiotic rubiflavin is in subclass II-2, and the very similar iyomycin B_1 is in subgroup III-1. Similarly, the sugar

containing chromomycine A_2 and olivomycin are in subgroup III-5, while the very similar gelbecidine is in subgroup IV-4.

A comparison between the results obtained using TLC classification for 150 antibiotics (8) and those obtained by instant TLC for 91 antibiotics (9), indicates the following: the main antibiotic classes were obtained in both systems by the same general procedures. Antibiotics not moving in any of the primary solvent systems formed group I; those which moved only with methanol formed group II; and those which moved with all primary solvents formed group IV in the ITLC system and group V in the TLC system. Those which moved in the solvents 10% methanol in chloroform and methanol formed group III in both systems, and those which moved with the previous two solvents and ethylacetate formed group IV in the TLC system. The TLC system has five and the ITLC system has four main antibiotic groups.

It is clear from the above results that the classification of antibiotics into different groups according to their mobility in a number of solvent systems does not clarify these antibiotics according to their chemical nature. Also migration of an antibiotic in more or less adsorbent does not reflect their primary structures.

CONCLUSION

The usefulness of the above presented TLC-bioautography classification systems was discussed in connection with screening for new antibiotics, analysis of pharmaceutical preparations, and identification of antibiotics in different samples. These samples could be crude preparations, physiological solutions, animal food additives, or antibiotic mixtures. The number of antibiotics classified by these TLC and ITLC systems is not too large (225), however, these systems can be extended according to needs to include the most important antibiotics in a par-

ticular laboratory. No single system can be designed for all known 4500 antibiotics originating from fermentations. The number of known antibiotics from other sources, i.e., animal tissues, plants, and marine, resources total over 2000 at this time. However, depending on the needs, the most important antibiotics can be included in the present system or a new system can be set up modeled after this system. It is our experience that the classification of over 200 antibiotics in one system may result in too large a number of subclasses with ambiguous results, i.e., same R_f values. However, combination of different methods, such as MS, IR, PC, gas and high performance liquid chromatography, and biological testing using microbes and mammalian cells, with TLC classification may increase the number of antibiotics that can be included in a classification system suitable to the needs of a particular laboratory. The use of computer storage and capability, we believe, is a tremendous asset for the present type of classification.

TLC is not only used for the separation, classification and quantitation of unknown antibiotics in a fermentation media, but to determine the optimum harvest time of antibiotics. For example, TLC was used to quantitatively determine the harvest time of verrucarin (20). Other uses of TLC in the antibiotic field were reviewed by Aszalos and Frost (9) and by Betina (15).

ACKNOWLEDGEMENT

One of us (HJI) was supported by Contract NO1-CO-73580 with the National Cancer Institute, NIH, Bethesda, Maryland 20205.

REFERENCES

1. N. Ishida, T. Shiratori, and S. Okamoto. J. Antibiot. (Series A) 3:880, 1950.
2. V. Betina. J. Chromat. 15:376, 1964.
3. V. Betina and P. Nemec. Nature 187:1111, 1960.
4. N. Snell, K. Ijichi, and J.C. Lewis. Appl. Microbio. 4:13, 1956.
5. H.J. Issaq, N.H. Risser, and A. Aszalos. J. Liquid Chromatogr. 2:533, 1979.
6. H.J. Issaq, J.A. Chan and E.W. Barr. J. Chromatogr. 152:280, 1978.
7. H.J. Issaq and E.W. Barr. Anal. Chem. 490:83A-96A, 1977.
8. H.J. Issaq, E.W. Barr, T. Wei, C. Meyers, and A. Aszolos. J. Chromat. 133:291, 1977.
9. A. Aszalos and D. Frost. In Methods in Enzymology (J.H. Hash, ed.), Vol. XLIII, Academic Press, New York. Thin layer chromatography of antibiotics. p. 172, 1975.
10. A. Aszalos, S. Davis, and D. Frost. J. Chromat. 37:487, 1968.
11. F. Kreuzig. J. Chromatogr. 142:444, 1977.
12. Y. Ito, N. Namba, N. Nagahoma, T. Yamaguchi, and T. Okuda. J. Antibiot. (Series A) 17:218, 1969.
13. J.P. Schmitt and C. Mathis. Ann. Pharm. France 23:205, 1970.
14. J.P. Schmitt and C. Mathis. Ann. Pharm. France 21:727, 1968.
15. V. Betina. J. Chromatogr. 78:41, 1973.
16. J.A. Chan, T.T. Wei, C.C. Kalita, D.J. Warnick, A.L. Garretson, and A. Aszalos. J. Antibiot. 30:1201, 1977.
17. A. Aszalos. Unpublished results.
18. R.J. Stroshane, J.A. Chan, E.A. Rubalcaba, A.L. Garretson, A.A. Aszalos, and P.P. Roller. J. Antibiot. 32:197, 1979.

19. M. Bostian, R. McNih, A. Aszalos, and J. Berdy. J. Antibiot. 30:663, 1977.

20. H.J. Issaq. Unpublished results.