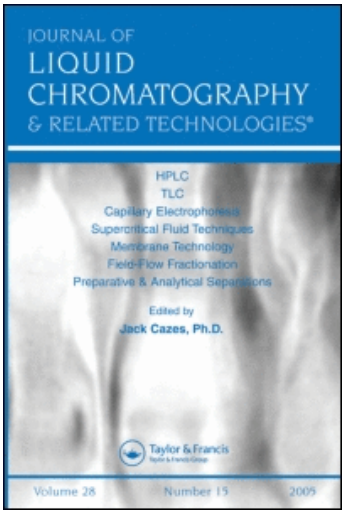

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Reversed-Phase Thin-Layer Chromatography of Synthetic Sulfonamide Antibacterials by Using Alkylsilyl Silica Gels

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REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY OF SYNTHETIC SULFONAMIDE ANTIBACTERIALS BY USING ALKYLSILYL SILICA GELS*

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ABSTRACT

Reversed-phase thin-layer chromatographic separation of ten kinds of synthetic sulfonamide antibacterials and related compounds was studied by using three kinds of chemically-bonded alkylsilyl silica gel (dimethylsilyl-, dodecylsilyl- and octadecylsilyl silica gels) as stationary phase and two kinds of organic solvents (methanol and dioxane) containing water as

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mobile phase. The compounds tested were sulfanilamide, sulfadiazine, sulfamerazine, sulfamonomethoxine, sulfadimethoxine, sulfamethoxazole, N^4-acetylsulfamethoxazole, sulfisoxazole, sulfathiazole and p-aminomethylbenzenesulfonamide. When dimethylsilyl silica gel containing 5% gypsum was used as stationary phase, methanol-water (1:3) with 0.005M 1-heptanesulfonic acid-acetic acid (pH 3.5) was the most effective for the separation of these antibacterials.

INTRODUCTION

Several authors have reported reversed-phase paper (1), reversed-phase thin-layer (RPTLC, 2) and colum (3) chromatographies of various organic compounds on silica gel, Kieselguhr and cellulose impregnated with non-polar stationary phases.

Recently, several kinds of chemically-bonded alkylsilyl silica gels have been prepared for reversed-phase high-performance liquid chromatography (RPHPLC) use (4), and subsequently been applied to RPTLC (5-8). The present authors have studied RPTLC separation of pharmceuticals such as cephalosporin antibiotics (9), steroidal hormones (10), and benzodiazepine minor tranquilizers (11) on dimethylsilyl-, octylsilyl- and octadecylsilyl silica gels. In this paper, the authors report RPTLC separation of ten kinds of synthetic sulfonamide anti-

bacterials and related compounds by using three kinds of alkylsilyl silica gel as stationary phase and two kinds of organic solvents containing water as mobile phase.

MATERIALS AND METHODS

Sulfonamides The compounds tested were sulfanilamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamonomethoxine, sulfamethoxazole, N^4-acetylsulfamethoxazole, sulfisoxazole, sulfathiazole and p-aminomethylbenzenesulfonamide. Table 1 shows the chemical and physical properties of these compounds. They were dissolved in methanol, except sulfamerazine in acetone, sulfisoxazole in a 1:1 mixture of methanol and chloroform, and sulfadiazine in a 1:1 mixture of 1N sulfuric acid and acetone. The amount of each compounds applied was 10μg per spot.

RPTLC stationary phases Merck silica gel 60 F_{254} silanised (RP-2, Art. 5747), Antec dodecylsilanised silica gel L_{254} (RP-12) and Whatman KC_{18} reversed-phase TLC with fluorescent indicator were used as precoated chromatoplates. For laboratory-prepared chromatoplate, Merck silica gel F_{254} silanised (RP-2, Art. 7750) and gypsum (Wako Chemicals, for TLC use) as a binder were used. Table 2 shows the properties of these stationary phases.

TABLE 1

Synthetic Antibacterials and Related Compounds

Sulfonamide	Chemical formula	MW	mp	λ_{max}^{MeOH} nm	$\varepsilon \times 10^4$	pKa
Sulfanilamide	$C_6H_8N_2O_2S$	172.21	165	263	1.90	10.4
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	250.28	254			6.4
Sulfamerazine	$C_{11}H_{12}N_4O_2S$	264.30	236			6.9
Sulfamonomethoxine	$C_{11}H_{12}N_4O_3S$	280.30	203	274	1.93	
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	310.33	202	272	2.20	6.0
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.31	167	270	1.93	1.4, 5.7
N^4-Acetylsulfamethoxazole	$C_{12}H_{13}N_3O_3S$	295.35	210	263	2.19	
Sulfisoxazole	$C_{11}H_{13}N_3O_3S$	267.30	194			4.9
Sulfathiazole	$C_9H_9N_3O_2S_2$	255.32	202	289	2.12	7.2
p-Aminomethylbenzenesulfonamide	$C_7H_{10}N_2O_2S$	186.25	152	225	1.21	

TABLE 2

Stationary Phases for RPTLC Use

Adsorbent	Dp(μm)[b]	Bristle	Silanised[c] carbon(%)	Manufacturer	Binder
Silica gel 60 HF_{254} silanised	10-40	C_2H_6	3.93	Merck	none[a]
TLC plates silica gel 60 F_{254} silanised pre-coated	10-40	C_2H_6	3.78	Merck	organic
KC_{18} reversed-phase TLC with fluorescent indicator	10	$C_{18}H_{37}$	14.27	Whatman	organic
Antecgel-Dodecyltrichlorosilan Opti-up C_{12} L_{254}	5-50	$C_{12}H_{25}$	3.39	Untec	inorganic

a) The laboratory-prepared plates with addition of 5% gypsum.

b) Particle diameter

c) Elemental analysis

Detection Sulfonamides spots were detected by using fluorescence quenching procedure or iodine vapor staining followed by Ehrlich's reagent.

Reagents For ion-pair RPTLC separation of the sulfonamides, Waters PIC B-7 (1-heptanesulfonic acid-acetic acid, pH 3.5) was added to methanol-water (1:3) mobile phase.

RESULTS AND DISCUSSION

RPTLC separation of sulfonamides

Mobile phase Two kinds of organic solvent containing water, methanol and dioxane, were used in ratios of (2:1), (1:1), (1:2) and (1:3).

Comparison of mobile phase Table 3 shows the RPTLC of sulfonamides separated with methanol-water and dioxane-water. It was found that the former system was superior to the latter in mean separation factor $\bar{\alpha}$, which was calculated from α=larger hR_f value/smaller hR_f value.

Comparison of mixing ratio Table 4 gives the RPTLC separation of sulfonamides by using four kinds of methanol-water mixture. In the separation in (2:1) and (1:1) mixtures, the hR_f values (R_f x 100) of the compounds became larger except p-aminomethylbenzenesulfonamide and their RPTLC separation was reduced. In the separation in (1:2) and (1:3) mixtures, mean separation factor $\bar{\alpha}$ of the compounds was nearly equal and the good resolution was obtained.

TABLE 3

RPTLC of Synthetic Sulfonamide Antibacterials and Related Compounds[a)]

	MeOH-H_2O (1:3)		Dioxane-H_2O (1:3)	
Compound	hR_f	α	hR_f	α
Sulfaniamide	66	2.13	63	1.50
Sufadiazine	31	1.29	42	1.20
Sulfamerazine	24	1.71	35	1.59
Sulfamonomethoxine	14^l	1.75	22^l	1.83
Sulfadimethoxine	8^l	2.38	12^l	1.58
Sulfamethoxazole	19^l	1.46	19^l	1.12
N^4-Acetylsulfamethoxazole	13^l	1.54	17^l	1.76
Sulfisoxazole	20^l	1.35	30^l	1.50
Sulfthiazole	27	1.93	45	1.50
p-Aminomethylbenzene-sulfonamide	14		30	
$\bar{\alpha}$		1.73		1.51

a) TLC condition; stationary phase, Merck silica gel 60 HF_{254} silanised with 5% gypsum detection, UV_{254} quenching followed by I_2 vapor;α=larger hR_f/smaller hR_f, $\bar{\alpha}$=mean α value, α > 1.2:separable, l:leading.

TABLE 4

RPTLC of Synthetic Sulfonamide Antibacterials and Related Compounds[a)]

	MeOH-H_2O (2:1)[b)]	(1:1)[b)]	(1:2)[c)]		(1:3)[c)]	
Compound	hR_f	hR_f	hR_f	α	hR_f	α
Sulfanilamide	90	80	69	1.60	66	2.13
Sulfadiazine	93	92	43	1.23	31	1.29
Sulfamerazine	90	85	35	1.75	24	1.71
Sulfamonomethoxine	93	93	20^1	1.67	14^1	1.75
Sulfadimethoxine	91	90	12^1	1.92	8^1	2.38
Sulfamethoxazole	92	93	23^1	1.53	19^1	1.46
N^4-Acetylsulfamethoxazole	94	93	15^1	2.33	13^1	1.54
Sulfisoxazole	95	92	35^1	1.14	20^1	1.35
Sulfthiazole	90	85	40	2.00	27	1.93
p-Aminomethylbenzene-sulfonamide	15	8	20		14	
$\bar{\alpha}$				1.69		1.73

a) TLC condition as in TABLE 3;stationary phase, b)pre-coated plate silica gel 60 F_{254} silanised, c)laboratory-prepared plate silica gel 60 HF_{254} silanised, 1:leading

Effect of addition of gypsum Table 5 shows the RPTLC separation of the sulfonamides on two kinds of laboratory-prepared chromatoplates. The laboratory-prepared plates with 5% gypsum was superior to that without gypsum in the spot compactness and mean separation factor $\bar{\alpha}$. The addition of gypsum gives a high packing density to the chromatoplates and does not hinder the RPTLC separation of these compounds.

Among three kinds of alkylsilyl silica gel, RP-2, RP-12 and RP-18, RP-2 gave better separation than the other two. As the degree of alkylsilylation of RP-12 stationary phase is low (Table 2, silanised carbon content 3.39%), it may be unable to affect the RPTLC separation of sulfonamides. The separation system, RP-2 stationary phase with 5% gypsum and 0.005M 1-heptanesulfonic acid-acetic acid (pH 3.5) in methanol-water (1:3), gives more compact spots with reduced tailing.

Detection As these sulfonamides have strong ultraviolet absorption (λ=260-290 nm, ε=19.000-22.000, Table 1), fluorescence quenching at 254 nm was useful for the detection of these compounds (detection limit, 0.03-0.1 μg per spot). Ehrlich's reagent (detection limit, 0.02-1.0 μg per spot) and iodine vapor were also effective. Table 6 shows the detection limits of these sulfonamides.

Comparison between separations with normal phase (NP) TLC and with RPTLC Table 7 shows the separation of sulfonamides in NPTLC and in RPTLC. Although separations

TABLE 5

RPTLC of Synthetic Sulfonamide Antibacterials and Related Compounds[a)]

	RP-2[b)]		RP-2 + 5% gypsum[b)]	
Compound	hR_f	α	hR_f	α
Sulfanilamide	66	2.13	67	1.97
Sulfadiazine	31	1.29	34	1.42
Sulfamerazine	24	1.71	24	1.42
Sulfamonomethoxine	14^l	1.75	17^t	2.13
Sulfadimethoxine	8^l	2.38	8	3.38
Sulfamethoxazole	19^l	1.46	27^t	1.13
N^4-Acetylsulfamethoxazole	13^l	1.54	24^t	1.58
Sulfisoxazole	20^l	1.35	38^t	1.19
Sulfathiazole	27	1.93	32	2.75
p-Aminomethylbenezenesulfonamide	14		88	
$\bar{\alpha}$		1.73		1.89

a) TLC condition as in TABLE 3;mobile phase, MeOH-H_2O(1:3) with PIC B-7.

b) laboratory-prepared plate. t;taling

TABLE 6
Detection of Synthetic Sulfonamide Antibacterials and Related Compounds[a)]

Compound	Limit of detection µg/spot		
	Fluorescence quenching[b)]	I_2 vapor	Ehrlich[c)]
Sulfanilamide	0.1	0.5	0.02
Sulfadiazine	0.1	0.1	0.05
Sulfamerazine	0.07	0.1	0.02
Sulfamonomethoxine	0.1	0.1	0.03
Sulfadimethoxine	0.05	0.05	0.03
Sulfamethoxazole	0.05	0.1	0.02
N^4-Acetylsulfamethoxazole	0.05	nd[e)]	0.1
Sulfisoxazole	0.05	0.1	0.02
Sulfathiazole	0.06	0.08	0.03
p-Aminomethylbenzenesulfonamide	nd[d)]	1.0	1.0

a) TLC condition as in TABLE 3. b) Inspected under UV irradiation at 254 nm.
c) p-Dimethylaminobenzaldehyde/HCl gas.
d) not detectable at 10µg. e) not detectable at 3µg.

TABLE 7

Comparison of RPTLC with NPTLC
in the Separation of Synthetic Sulfonamide Antibacterials[a)]

Substituent group		Difference in hR_f		Solvent system		
		RPTLC	NPTLC	RPTLC	NPTLC	
$-CH_2-$	Sulfanilamide and p-Aminomethyl-benzenesulfonamide	52	37	MeOH-H_2O (1:3)	EtOAc Benzene MeOH NH_4OH	5 1 2 1
$-CH_3$	Sulfadiazine and Sulfamerazine	7	10	MeOH-H_2O (1:3)	"	"
$-COCH_3$	Sulfamethoxazole and N^4-Acetylsulfa-methoxazole	6	36	MeOH-H_2O (1:3)	EtOAc Benzene	3 1
$-OCH_3$	Sulfamonomethoxine and Sulfadimethoxine	10	15	Dioxane-H_2O (1:3)	"	"

a) RPTLC condition as inTABLE 3, NPTLC condition;stationary phase, Merck pre-coated TLC plates silica gel 60 F_{254}.

of the same magnititude were obtained, complex mobile phase and prolonged development were necessary for NPTLC. RPTLC requires rather simple mobile phase components and mixing ratios, and its wide application to pharmaceutical analysis will be promised.

CONCLUSION

The RPTLC separation of synthetic sulfonamide antibacterials was effected by using dimethylsilyl silica gel (RP-2) with 5% gypsum as stationary phase and methanol-water (1:3) containing 1-heptanesulfonic acid.

The RPTLC with chemically-bonded alkylsilyl silica gels as stationary phase seems to have wide application in the separation of various pharmaceuticals including sulfonamide antibacterials, benzodiazepine minor tranquilizers, steroidal hormones and cephalosporin antibiotics.

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