Journal of Chromatography, 291 (1984) 249–256
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,576

COMPARISON OF SILICA-GEL AND REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY IN THE TESTING OF DRUGS

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(First received October 4th, 1983; revised manuscript received January 13th, 1984)

SUMMARY

In identity and purity testing of 28 drug substances we compared the effectiveness of combinations of an acidic and a basic mobile phase chosen from five universal halogen-free systems, most of them described in an earlier paper [*Z. Anal. Chem.*, 295 (1979) 398] with four widely used mobile phases described by Stead *et al.* [*Analyst (London)*, 107 (1982) 106] in thin-layer chromatography (TLC) on silica gel. We also employed reversed-phase TLC on C_{18} bonded phases, using acidic and basic mobile phases, and found the two methods to be of similar versatility and complementary suitability.

With regard to TLC on silica gel, our universal mobile phases have a much higher separating power than similar mobile phases used for high-performance liquid chromatography on silica gel. The methods are compared, and practical approaches are discussed for the optimization of the reversed-phase TLC technique.

INTRODUCTION

The literature on the use of silica-gel thin-layer chromatography (TLC) for drugs has reached an enormous extent. Stead *et al.*[1] chose (mainly from drug identification literature) the 29 most popular and promising mobile phases and tested them with 100 basic and 85 neutral and acidic drugs. They selected the best four (one with chloroform) for testing basic drugs on silica gel treated with potassium hydroxide, and the four most effective for testing acidic and neutral drugs. The latter group consisted of the following: ethyl acetate–methanol–ammonia (85:14:1); chloroform–methanol (9:1); chloroform–acetone (4:1); ethyl acetate.

In our opinion, chloroform should be avoided as far as possible for environmental reasons. In a previous paper[2] we described five halogen-free mobile phases for efficient TLC of 151 out of 157 basic, acidic and neutral drugs; no potassium hydroxide treatment of the TLC plates was necessary. Using the same mobile phases, with one addition, we have now compared their separating powers with those of the four mentioned above, for acidic and neutral drugs.

There is much less literature available on bonded-phase reversed-phase (RP)

0021-9673/84/$03.00

TLC, although a review by Brinkman and De Vries[3] contained 93 references. In this study of 28 drug substances we also tested acidic, basic and neutral mobile phases similar to those most commonly used for RP high-performance liquid chromatography (HPLC).

The main objectives of this paper are to compare the performances in identity and purity testing of RP and silica-gel TLC, and to judge the predictability of HPLC mobile phases from TLC values. Furthermore, we wanted to demonstrate that combination of the TLC techniques gives TLC an edge over HPLC, because TLC offers the following advantages:

From the start line to the solvent front nearly everything is detectable.

Iodine chamber and a great number of specific detection reagents can be used, and non-UV-absorbing substances can easily be detected.

Many substances or many samples, as well as reference substances, can be chromatographed in one run.

Dirty or turbid samples can be analysed.

UV-absorbing or corrosive mobile phases can be applied.

It is simple and cheap.

Elaboration is fast and easy.

On the other hand, the advantages of HPLC are mainly in the field of assays:

Assays can be fully automated.

Assays are more accurate than quantitative TLC.

Calibration curves are usually linear, in contrast to TLC[4].

Resolution is better.

UV sensitivity is better.

Substances sensitive to light or oxygen give less problems than with TLC.

EXPERIMENTAL

Working technique for RP-TLC

Application. To obtain the values listed in the tables we used 0.5 μl (half of a 1-μl Drummond Microcap) of 2 g per 100 ml solutions in methanol*. After application the plates were dried at 50°C for 30 min, quickly cooled on a plate and put into the tank within 1 min. In contrast to TLC on silica gel, drying of the RP plates had only a minor influence. Plates for purity testing were not dried at elevate temperatures in order to avoid any decomposition or oxidation.

Starting line. This was either 20 mm above the edge or 10 mm above the mobile phase.

Developing chamber. Normal tank with 100 ml (1 cm in height) of mobile phase. (For routine work double-trough chambers needing only 20 ml of mobile phase may be used); no chamber saturation and no paper wicks were applied.

Mobile phases. All percentage data in the tables are in terms of volume per cent.

* In routine work the usual sample amounts were: 0.1–1 μg for quantitative TLC; 1–20 μg for identification; *ca.* 100 μg for purity testing. Using 1 μg in 0.5 μl, the spot diameter after development usually is less than 5 mm; using 10 μg, *ca.* 6 mm; and using 100 μg, *ca.* 12 mm. For identification we always use reference substances.

Length of run. This was 6 cm, requiring 20–30 min at *ca.* 20°C, except mobile phases containing 2-propanol, which needed *ca.* 45 min.

Detection. UV detection at 254 nm and an iodine chamber (1 h) were used.

Plates. To obtain the values in the tables, we used Whatman KC 18 F plates, 20 × 20 cm, cut to 10 × 20 cm. About fifteen samples were applied.

Working technique for silica-gel TLC

We worked as described for RP-TLC except that we used Merck TLC plates silica gel 60 F_{254} (Art. 5715).

TABLE I

hR_F VALUES ON SILICA GEL 60 F_{254} MERCK

Mobile phases: A = toluene–ethyl acetate–formic acid 85% (50:45:5); B = toluene–2-propanol–conc. ammonia (70:29:1); C′ = toluene–acetone–2 *N* acetic acid (30:65:5); D = toluene–2-propanol–ethyl acetate–2 *N* acetic acid (10:35:35:20); E = toluene–dioxane–methanol–conc. ammonia (20:50:20:10). F = front, T = tailing. a = Acid, b = base, n = neutral substance.

Substances	*Type*	*Mobile phases*				
		A	*B*	*C′*	*D*	*E*
Benzoic acid	a	68	3	72	F	23
Bepridil hydrochloride	b	7	84	23	46	F
Chlorzoxazone	n	66	52	83	95	43
Dienestrol	n	73	67	83	94	80
Difenoxin hydrochloride	ab	28	7	41	83	46
Econazole nitrate	b	4	71	69	79	89
Edurid	n	6	22	42	75	55
Estriol	n	26	47	57	87	66
Ethinyl estradiol	n	69	70	82	92	83
Haloperidol	b	3	58	14–26	55	95
Mestranol	n	74	80	91	94	95
Methylparaben	n	68	62	83	F	65
Miconazole nitrate	b	6	71	71	81	94
Moperone hydrochloride	b	6	54	13–28	56	95
Norethisterone	n	59	72	85	94	86
Paracetamol	n	34	41	61	86	61
Propylparaben	n	74	60	90	F	69
Sulfabenzamide	a	54	3	75	95	34
Sulfacetamide	a	33	2	69	87	20
Sulfanilamide	n	30	34 T	66	86	63
Sulfapyridine	n	28	20	71	87	47
Sulfathiazole	a	17	4	58	80	31
Suprofen	a	68	3	74	94	30
Terconazole	b	St	56	5	18	95
Tolmetin sodium dihydrate	a	60	3	67	90	28
Triamcinolone	n	34	57	60	89	62
Triamcinolone acetonide	n	17	59	70	87	73
Zomepirac sodium dihydrate	a	61	2	68	91	30

RESULTS AND DISCUSSION

TLC on silica gel

We used the universal halogen-free TLC mobile phases A, B, D, and E described in our earlier paper[2]. However, instead of C (toluene–2-propanol, 90:10) we used an additional universal mobile phase C' (see Table I). Analysis of the TLC data in Table I in our earlier paper showed that the acidic mobile phase A and the basic mobile phase E were the most efficient for the 35 acidic and 47 neutral drug substances; B and D were best for the 69 basic drug substances.

In Table I of this paper 22 of the 28 drug substances investigated are acidic or neutral (including the amphoteric difenoxin). We obtained superior TLC results for the 22 substances with mobile phases A and E compared with results obtained using the four mobile phases of Stead *et al.* mentioned above: the number of acceptable spots between R_F 10 and 90 with A and E combined was 42 out of 44, whereas with the first two of Stead's combined we obtained 32 out of 44 and with the third and fourth combined only 11 out of 44. With hR_F 6 as a window value, we obtained with A and E combined a discriminating power* of 0.98, compared with 0.96 for the best combination from Stead's four mobile phases.

Reversed-phase TLC

In our experience, the combination of an acidic and a basic mobile phase in RP-TLC enhances the separation probability (especially for basic substances) compared with the use of two neutral mobile phases. In purity testing the best combination of mobile phases for the 22 acidic and neutral substances (Table II) was IV and V, giving 40 acceptable spots out of 44.

Comparison of RP and silica-gel TLC

The strong retention of ions on silica gel means that a strongly polar basic mobile phase and a relatively weakly polar acidic mobile phase are needed for the TLC of acids, and that a strongly polar acidic mobile phase and a relatively weakly polar basic mobile phase are needed for the TLC of bases. In RP-TLC the ionization effect is reversed and much weaker. Furthermore, the differences between the R_F values obtained with mobile phases of similar eluotropic strength are much lower than on silica gel. Therefore in identity testing by RP-TLC the use of more than two mobile phases does not bring much additional information.

With RP-TLC it is very easy to find a good mobile phase, because there are only a few volatile water-miscible solvents available, and most of them are generally suitable. On the other hand, the wider variety of possible good mobile phases in silica-gel TLC increases the separation potential.

During purity testing we found 31 impurities in the 22 acidic and neutral substances on silica gel with the mobile phases A + E, and 36 impurities with the RP mobile phases IV and V. Only 21 of the total of 44 impurities revealed by using all four mobile phases were found on silica gel as well as on the bonded phase. It must be considered that, on silica gel, separation is due mainly to differences in the polar-

* The discriminating power for a combination of two mobile phases is the probability (between 0 and 1) that any two substances can be separated in at least one of the two mobile phases[5].

TABLE II

RP, hR_F VALUES ON WHATMAN KC 18 F

Mobile phases: I = methanol–0.5% phosphoric acid–3% sodium chloride (60:10:30); II = 2-propanol–0.5% phosphoric acid–3% sodium chloride (40:10:50); III = 2-propanol–methanol–0.5% phosphoric acid–3% sodium chloride (23:23:10:44); IV = tetrahydrofuran (THF)–methanol–0.5% phosphoric acid–sodium chloride (28:28:10:34); V = methanol–2 *N* ammonia–3% sodium chloride (60:10:30); VI = 2-propanol–2 *N* ammonia–3% sodium chloride (40:10:50); VII = 2-propanol–methanol–2 *N* ammonia–3% sodium chloride (23:23:10:44); VIII = THF–methanol–2 *N* ammonia–3% sodium chloride (28:28:10:34). F = front, T = tailing, St = start.

Substances	*Mobile phases*							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
Benzoic acid	78	67	73	70	87	85	83	93
Bepridil hydrochloride	5	22	12	26	St	St	St	St
Chlorzoxazone	32	35	33	32	60	47	43	55
Dienestrol	9	28	17	13	10	28	12	19
Difenoxin hydrochloride	9	St	St	36	19	35	23	46
Econazole nitrate	St	5	St	6	St	3	St	7
Edurid	84	83	86	83	88	82	83	88
Estriol	30	48	42	44	33	45	22–42	50
Ethinyl estradiol	9	29	18	17	11	28	17	22
Haloperidol	28	38	31	39	4	12 T	8	14
Mestranol	St	8	3	7	St	12 T	St	8
Methylparaben	44	45	44	42	60	53	57	58
Miconazole nitrate	St	2	St	3	St	St	St	4
Moperone hydrochloride	33	41	35	51	9	13	12	15
Norethisterone	11	28 T	15 T	28	19	30	0–22	27
Paracetamol	79	75	80	74	86	72	76	77
Propylparaben	21	30	22	28	33	38	38	34
Sulfabenzamide	79	76	78	70	89	83	84	88
Sulfacetamide	90	89	91	80	F	F	95	F
Sulfanilamide	92	91	93	82	F	88	87	81
Sulfapyridine	78	80	79	70	84	83	82	77
Sulfathiazole	84	81	83	77	90	85	87	89
Suprofen	48	55	51	54	67	60	60	71
Terconazole	13	41	27	42	St	7	3	12
Tolmetin sodium dihydrate	42	56	48	60	48	53	50	67
Triamcinolone	45	61	55	59	45	60	57	57
Triamcinolone acetonide	18	43	32	38	21	43	29	41
Zomepirac sodium dihydrate	33	48	37	44	40	48	41	56

ities of the substances; on RP plates solubility in the mobile phases is influential, as well as structural differences in the non-polar part of the molecule. In purity testing it is therefore advisable to use both TLC methods.

It should still be emphasised however that the costs of the commercial RP-TLC plates are considerably higher than those of the silica gel plates.

Discussion of RP-TLC optimizations in identity and purity testing of drugs

For unknown substances we recommend starting with mobile phases IV and V of Table II. If the hR_F values are too low or too high, the concentration of the organic solvent must be adjusted according to the empirical rule that a reduction of

TABLE III

RP hR_F VALUES OF NEUTRAL SUBSTANCES ON WHATMAN KC 18 F

Mobile phases: 1 = acetonitrile–3% sodium chloride (50:50); 2 = methanol–3% sodium chloride (60:40); 3 = acetone–3% sodium chloride (50:50); 4 = THF–3% sodium chloride (43:57); 5 = 2-propanol–3% sodium chloride (40:60); 6 = methanol–2-propanol–THF–3% sodium chloride (17:17:17:49). F = front, T = tailing, St = start.

Substances	*Mobile phases*					
	1	*2*	*3*	*4*	*5*	*6*
Chlorzoxazone	46	32	25	18	35	36
Dienestrol	25	11	7	10	29	21
Estriol	61	29	40 T	34	35–50	48 T
Ethinyl estradiol	30	11	11	11	29	23
Methylparaben	51	42	35	25	42	44
Mestranol	8	St	3	5	2–13	10
Norethisterone	25	12	7–17	16	15–23	30
Paracetamol	68	80	75	59	73	75
Propylparaben	33	20	17	15	28	29
Sulfanilamide	68	93	77	58	85	81
Triamcinolone	65	44	53	40	61	61
Triamcinolone acetonide	45	19	26	26	42	44

10 ml of methanol per 100 ml decreases the hR_F value by *ca.* 10–20. For neutral substances the neutral mobile phases listed in Table III may be used, but we usually avoid the toxic acetonitrile. To solve special selectivity problems, dioxane and ethanol as well as mixtures might also be used. (The mixture of THF with methanol was better than THF alone.)

Sodium chloride in the mobile phase enhances the wettability of the plates and is needed in particular when the water content exceeds *ca.* 20%. Considerable differences in separation may be obtained if sodium chloride is not added. A water content exceeding *ca.* 60% usually gives bad spot shapes.

Brinkman and De Vries[6] compared eight types of commercially available RP-TLC plates. One of their conclusions is that the different behaviour of the eight types increases the potential of the technique as a tool for separation. We have used Merck RP-18 and Whatman KC 18 plates, and both have advantages. Using spray reagents as well as UV detection and an iodine chamber, we obtained better sensitivities with Whatman KC 18 because of the lighter plate background (especially in UV light for spots near the front).

TLC and HPLC on silica gel

Most of the versatile and efficient TLC mobile phases for silica gel containing toluene, acetone, or ethyl acetate cannot in most cases be used in silica-gel HPLC because of their UV-absorbing properties. Thus only the solvents *n*-hexane or dichloromethane can be used as the main component, with added methanol, 2-propanol, or THF. The resulting TLC mobile phases are relatively inefficient, and their versatility is limited. The number of acceptable spots, with hR_F values, ranging from

TABLE IV

COMPARISON OF RETENTION CAPACITY FACTORS (k') IN HPLC WITH hR_F VALUES IN TLC

k' values are calculated using the methanol peak as zero retention, $k' = 0$. Retention times in minutes for SPH: 1.3 + 1.3 k'; for Bond: 2.8 + 2.8 k'. SPH = Spherisorb ODS, 5 μm, 125 × 4.6 mm I.D. Bond = Micro-Bondapak C_{18}, 300 × 4 mm I.D. Whatman = KC 18 F RP-TLC plates 20 × 20 cm, 0.20 mm, No 4803-800. Merck = RP-18 F_{254} S plates 10 × 20 cm, 0.25 mm, No 15423.

Substances	*Methanol 60% (v/v)*				*Acetonitrile 50% (v/v)*		
	RP-HPLC		*RP-TLC*		*RP-HPLC*	*RP-TLC*	
	k' *SPH*	k' *Bond*	hR_F *Whatman*	hR_F *Merck*	k' *SPH*	hR_F *Whatman*	hR_F *Merck*
Sulfanilamide	0.1	0.0	93	88	0.2	70	62
Paracetamol	0.2	0.1	80	72	0.2	71	62
Triamcinolone	0.6	0.6	44	38	0.3	65	58
Methylparaben	0.6	0.6	42	35	0.6	47	42
Chlorzoxazone	1.1	1.0	32	23	0.9	42	34
Estriol	1.2	1.0	29	22	0.5	58	47
Propylparaben	1.6	1.8	20	16	1.4	32	25
Triamcinolone acetonide	1.8	2.0	19	13	1.1	43	33
Dienestrol	3.5	4.4	11	7	2.6	22	17
Ethinyl estradiol	4.1	4.1	11	7	2.2	26	17
Norethisterone	4.3	3.3	12	7	2.6	22	14
Mestranol	16.5	19.5	0	0	7.9	8	4

10 to 90, with the four best mobile phases* was six times less than with the four best TLC mobile phases in Table I. Therefore, the use of silica-gel TLC as a pilot technique for finding silica-gel HPLC mobile phases can only have restricted application.

Reversed-phase TLC and reversed-phase HPLC

Table IV compares RP-TLC and RP-HPLC data for neutral substances, obtained using methanol and acetonitrile. The table shows the well-known fact that C_{18} HPLC columns from different suppliers have different selectivities. The same is true for different makes of RP-TLC plates.

Table V gives a rough idea of what k' value may be expected for a known hR_F value when the same neutral mobile phase is used. (We have not yet investigated acidic and basic mobile phases.) The values show a good linearity using the equation of Geiss[7], and a value of 0.5 for k_f:

$$k' = k_f \left[\frac{1}{R_F} - 1 \right]$$

where k_f is the transference factor of TLC to HPLC.

* Dichloromethane–methanol–acetic acid (90:8:2); *n*-hexane–THF–acetic acid (25:73:2); dichloromethane–THF–ammonia (60:39:1); *n*-hexane–THF–ammonia (25:74:1).

TABLE V

RP-HPLC PREDICTIONS FROM RP-TLC VALUES, TESTED WITH NEUTRAL SUBSTANCES ACCORDING TO TABLE IV

TLC hR_F	83	66	50	32	18	10	5	0
HPLC k'	0.1	0.25	0.5	1	2	4	8	16

To obtain k' values between 1 and 4 in HPLC, it is necessary to use mobile phases that give hR_F values between 10 and 30 in TLC.

As an empirical rule for practical work, we found for methanol–water mixtures that decreasing the methanol concentration by 10 ml per 100 ml of mixture (i) roughly doubles k' in RP-HPLC, (ii) reduces the hR_F values in RP-TLC by *ca.* 10–20.

It is certainly possible that two substances with the same hR_F value could be separated by HPLC owing to a slightly different selectivity or the better resolution of HPLC.

In HPLC purity testing, low levels of impurities with high retention may not be detectable owing to peak broadening. On the other hand, RP-TLC gives the most sensitive or sharpest spots with highly retained substances. Furthermore, in HPLC impurities with very low retention may appear unresolved near $k' = 0$, but in TLC they may separate between *ca.* hR_F 50 and 90. Even impurities at the startline or the solvent front can be detected.

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