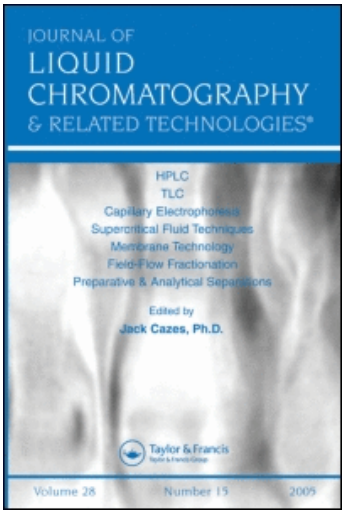

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Combined Use of Normal and Reversed Phase Thin Layer Chromatography in the Screening for Basic and Quaternary Drugs

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To cite this Article Ojanperä, Ilkka , Vartiovaara, Juhani , Ruohonen, Aira and Vuori, Erkki(1991) 'Combined Use of Normal and Reversed Phase Thin Layer Chromatography in the Screening for Basic and Quaternary Drugs', Journal of Liquid Chromatography & Related Technologies, 14: 8, 1435 — 1446
To link to this Article: DOI: 10.1080/01483919108049625
URL: http://dx.doi.org/10.1080/01483919108049625

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(8), 1435-1446 (1991)

COMBINED USE OF NORMAL AND REVERSED PHASE THIN LAYER CHROMATOGRAPHY IN THE SCREENING FOR BASIC AND QUATERNARY DRUGS

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ABSTRACT

The combined use of normal and reversed phase thin layer chromatography in drug screening is evaluated by the mean list length method. A reversed phase system, involving RP-18 plates and aqueous hydrochloric acid - methanol as a mobile phase, is shown to be an effective complementary pair to basic medium-polar normal phase systems. With a set of 140 basic and quaternary drugs, a mean list length of 1.8 is obtained for a TLC/RPTLC pair. The combination is also applicable to hydrophilic drugs extracted as bis(2-ethylhexyl)phosphate ion-pairs.

INTRODUCTION

The concept of using a combination of normal phase (TLC) and reversed phase (RPTLC) thin layer chromatographic systems in connection with ion-pair extraction for the

screening of basic and quaternary drugs was recently introduced by the authors (1). In order to include very hydrophilic drugs in the screen, a lipophilic acid, bis(2-ethylhexyl)phosphoric acid (HDEHP) (2-6), was used as a counter-ion in the extraction. The chromatographic systems were chosen to meet two basic requirements. Firstly, the combination should cover a wide drug polarity scale. Secondly, the interference caused by HDEHP present in the extracts should be minimal. A standard basic normal phase system and a reversed phase system developed for the purpose were found to fulfill these conditions (1).

The combined use of several TLC systems is an established practice in systematic toxicological analysis (7). However, the method described by the authors (1) was among the first to utilize the combination of TLC and RPTLC in that context. Recently, another combined TLC/RPTLC method, based on Toxi-Grams Blank A and C_8, has been described and evaluated (8).

In the present study, the RPTLC system (1) and a new TLC system (TLC_1) are characterized with 141 basic and quaternary drugs that are apparently extractable as HDEHP ion-pairs. This combination and a combination of the RPTLC system and a standard TLC system (TLC_2) with literature values (7), as well as the TLC_1/TLC_2, are evaluated using the mean list length (MLL) approach (9).

EXPERIMENTAL

Drug standards: For each drug, a solution of 2 mg/ml in methanol was prepared and 2 µl of the solution was applied to the plate. Seventeen samples were applied to each plate, leaving the outermost places empty.

Thin layer chromatographic plates: Precoated plates RP-18 $F_{254}s$ (15423, Merck, Darmstadt, FRG) and Silica gel 60 F_{254} (5554, Merck) were used in the size 10 cm x 20 cm. The RP plates were dryed according to manufacturer's directions prior to use.

Development: All plates were developed for 7 cm in a double trough developing tank (Camag, Muttenz, Switzerland), one plate at a time. For RP plates, 10 ml of the mobile phase, methanol - water - conc. HCl 50 + 50 + 1 (1), was put in a single trough, and the plate was developed directly. For silica gel plates, 15 ml of the mobile phase, toluene - acetone - ethanol - ammonia 45 + 45 + 7 + 3 (TLC_1) was divided equally into the troughs equipped with two pieces of filter paper, and the tank was allowed to saturate for 0.5 hours before development.

Detection: The plates were viewed under 254 nm and 366 nm UV light. Fast Black K salt reagent (10) and acidified iodoplatinate reagent were used when necessary.

Collection of Data: The migration distances were measured from four independent developments during a six-month period with an accuracy of 1 mm. The lot of TLC plates was changed twice during the study whereas the RPTLC plates were of same lot. The eluents were prepared separately for each development. In the RPTLC reproducibility study, the lot of RPTLC plates was different in each four development, and different from the lot used in Table 1. The eluent was prepared separately for each development.

Data Processing: The list length (L) and the MLL for systems and for combinations of systems were calculated according to the formulae described earlier: with the compounds' individual standard deviation (SD) values, the equation of normal distribution was used (ref.9, eq.1); with a constant SD for each system, the corrected equation was

TABLE 1

Chromatographic Detection Characteristics of Test Drugs

Drug	RPTLC			TLC_1			RPTLC/TLC_1
	hR_f	SD	L_{95}	hR_f	SD	L_{95}	L_{95}
Acebutolol	43	1	6	11	1	10	2
Acetophenazine	17	2	13	18	1	9	2
Ajmaline	39	2	6	29	1	17	4
Alcuronium	29	2	9	0	0	11	1
Alprenolol	21	1	12	27	1	14	2
Amantadine	42	1	7	11	1	10	2
Amfepramone	48	2	8	75	1	4	1
Amiloride	55	1	9	4	1	5	1
Amitriptyline	9	1	17	46	2	10	1
Amphetamine	57	1	9	37	2	14	1
Astemizole	19	1	15	40	2	12	3
Atenolol	77	1	6	4	0	5	1
Atropine	50	2	6	5	1	6	1
Biperiden	11	1	17	79	1	4	1
Bupivacaine	23	1	11	73	2	6	1
Buprenorphine	17	2	13	72	1	5	1
Butylscopolammmonium	34	2	8	0	0	11	1
Carbachol	93	1	3	0	0	11	2
Chloroquine	48	1	8	14	2	9	1
Chlorpromazine	5	1	9	50	2	10	2
Chlorprothixene	4	1	8	56	3	12	2
Cimetidine	76	2	6	15	1	8	2
Cinnarizine	15	1	12	78	1	4	1
Clemastine	4	1	8	30	2	14	2
Clomipramine	5	1	9	49	1	10	2
Clonidine	56	1	8	53	1	13	1
Clozapine	49	1	6	36	1	14	1
Cocaine	33	1	8	66	1	8	2
Codeine	66	2	8	16	1	9	1
Cyclizine	43	1	6	46	1	10	1
Debrisoquine	49	1	6	0	1	11	1
Dexchlorpheniramine	59	1	8	20	2	12	2
Dextromethorphan	18	1	13	21	2	12	3
Dextropropoxyphene	11	1	17	67	2	8	1
Dibenzepin	27	2	9	33	2	15	2
Diltiazem	13	1	15	42	2	11	3
Dimethindene	54	1	8	22	1	16	1
Diphenhydramine	20	1	14	39	2	11	3
Diphenylpyraline	15	1	12	28	1	16	1
Dipyridamole	9	1	17	36	2	14	3
Disopyramide	61	2	6	30	1	14	1
Dixyrazine	18	1	13	33	2	15	2
Doxepin	16	1	14	44	2	10	2
Emepronium	16	1	14	1	1	12	2
Ephedrine	63	0	8	7	1	6	1
Ethylmorphine	55	2	9	17	1	9	1
Etodroxizine	18	1	13	35	1	14	2

TABLE 1 continued

Drug	RPTLC			TLC_1			RPTLC/TLC_1
	hR_f	SD	L_{95}	hR_f	SD	L_{95}	L_{95}
Fencamfamin	25	1	11	57	2	12	4
Fenfluramine	27	1	9	33	3	15	2
Fenoterol	73	2	6	11	1	10	1
Flecainide	21	1	12	27	1	14	2
Flupenthixol	6	1	10	32	2	14	3
Fluphenazine	8	1	12	29	2	17	2
Glycopyrronium	18	1	13	0	1	11	2
Haloperidol	13	2	15	53	1	13	2
Hydralazine	75	1	7	68	2	8	1
Hydroxychloroquine	53	1	8	13	2	10	2
Hydroxyzine	20	1	14	41	3	12	3
Imipramine	11	1	17	40	1	12	3
Labetalol	28	1	8	21	0	12	1
Levomepromazine	7	1	15	60	1	10	2
Lidocaine	46	1	6	66	2	8	2
Maprotiline	9	1	17	11	1	10	2
Meclozine	6	1	10	81	2	3	1
Melperone	28	2	8	57	3	12	5
Mepivacaine	42	1	7	55	1	13	1
Mepyramine	68	1	6	37	2	14	1
Mescaline	63	2	8	26	1	15	3
Metformin	92	2	4	0	0	11	2
Methadone	9	1	17	54	3	14	3
Methamphetamine	53	1	8	12	1	11	2
Metoclopramide	39	1	6	25	2	13	2
Metoprolol	41	1	8	21	1	12	1
Mexiletine	34	2	8	47	2	10	1
Mianserin	17	1	13	50	3	10	2
Minoxidil	47	1	7	3	0	5	1
Moperone	16	2	14	50	1	10	2
Morphine	81	2	5	8	1	9	2
Nalorphine	73	1	6	30	3	14	1
Neostigmine	60	2	6	0	0	11	1
Nialamide	60	1	6	15	1	8	1
Nifedipine	22	1	11	61	1	10	1
Nortriptyline	9	1	17	20	1	12	1
Noscapine	34	2	8	69	2	8	1
Opipramol	23	2	11	20	1	12	2
Orciprenaline	89	2	5	6	1	7	1
Orphenadrine	13	1	15	45	2	10	2
Oxprenolol	32	1	9	21	1	12	1
Oxycodone	64	1	8	54	2	14	1
Oxypertine	27	2	9	61	2	10	3
Penfluridol	1	1	3	72	2	5	2
Pentazocine	31	2	12	55	1	13	2
Periciazine	12	2	12	36	2	14	2
Perphenazine	11	1	17	27	2	14	1
Pethidine	34	2	8	34	2	15	1
Pheniramine	76	2	6	18	3	9	1

(continued)

TABLE 1 continued

Drug	RPTLC			TLC_1			RPTLC/TLC_1
	hR_f	SD	L_{95}	hR_f	SD	L_{95}	L_{95}
Phentermine	47	0	7	21	1	12	1
Phenylpropanolamine	67	1	6	47	0	10	1
Pilocarpine	76	1	6	25	1	13	1
Pimozide	5	1	9	56	2	12	2
Pindolol	58	1	9	21	1	12	2
Pitofenone	26	2	10	56	1	12	4
Practolol	71	2	4	8	1	9	1
Prazosin	23	1	11	43	1	10	1
Prilocaine	47	1	7	64	2	9	2
Procainamide	92	3	4	13	1	10	1
Procaine	88	1	4	50	2	10	1
Prochlorperazine	10	0	18	31	1	14	3
Prolintane	28	2	8	60	3	10	3
Promazine	10	1	18	36	2	14	3
Promethazine	11	1	17	41	1	12	3
Propafenone	11	1	17	58	tailing		
Propranolol	21	1	12	24	1	11	1
Protriptyline	11	1	17	10	1	11	2
Quinidine	66	2	8	24	1	11	3
Quinine	66	2	8	24	1	11	3
Ranitidine	84	1	6	10	1	11	2
Reserpine	5	1	9	67	2	8	1
Salbutamol	83	1	6	6	1	7	2
Scopolamine	53	1	8	0	0	11	2
Sotalol	82	2	5	13	0	10	1
Sulpiride	71	0	4	17	1	9	1
Suxamethonium	86	2	7	0	0	11	1
Terbutaline	84	1	6	8	1	9	4
Tetracycline	44	0	6	0	0	11	1
Thiethylperazine	6	0	10	33	2	15	2
Thioproperazine	19	2	15	21	1	12	3
Thioridazine	3	1	9	45	2	10	1
Thiothixene	17	1	13	24	2	11	2
Timolol	40	1	7	27	1	14	2
Tocainide	55	2	9	42	1	11	1
Trazodone	23	1	11	53	2	13	1
Triamterene	40	1	7	10	1	11	2
Trifluoperazine	8	1	12	31	2	14	3
Trimeprazine	9	1	17	59	2	10	3
Trimipramine	9	0	17	65	2	9	1
Triprolidine	64	1	8	25	2	13	4
Tubocurarine	55	2	9	0	0	11	2
Verapamil	12	1	12	57	2	12	1
Zopiclone	35	2	7	30	1	14	3
Zuclopenthixol	1	1	3	78	3	4	1
MLL							
95%		9.70			10.88		1.79
99%		12.09			13.85		2.26
n		141			140		140

TABLE 2

Comparison of MLL Values for Two-System Combinations

	TLC_1/RPTLC	TLC_2/RPTLC	TLC_1/TLC_2
MLL A			
95%	1.74	2.50	5.52
99%	2.59	4.01	8.91
MLL B			
95%	3.97	4.10	7.44
99%	9.84	8.85	11.98
n	140	116	116

RPTLC: Methanol-Water-HCl 50+50+1
TLC_1: Toluene-Acetone-Ethanol-NH_3 45+45+7+3
TLC_2: Ethyl acetate-Methanol-NH_3 85+10+5
A: calculated on the basis of the following constant SDs: SD = 1.3 for RPTLC; SD = 1.7 for TLC_1; SD = 2.7 for TLC_2
B: calculated on the basis of a constant SD of 2.5 for all systems

used (ref.9, eq.2 and 3). In the case of SD = 0, a value of 0.5 was used. The calculations were carried out with an IBM AT compatible micro computer using a program written in Turbo Pascal by the authors. The program required no extra memory and accomplished a MLL calculation for 141 compounds in a few minutes.

RESULTS

Table 1 shows the mean hR_f values and their standard deviations for 141 drugs analysed by the RPTLC system (1) and the TLC_1 system. The table also shows the L values of individual drugs, calculated for the chromatographic systems and for their combination with 95% cumulative probability. The MLL values are presented with both 95% and 99% cumulative probability. Propafenone is not included in the results involving the TLC_1 because it produces a tailing spot on that system.

TABLE 3

Effects of R_f Correction on RPTLC Lot-to-lot Reproducibility

Drug	hR_f from Table 1	Uncorrected hR_f	Uncorrected SD	Corrected hR_f	Corrected SD
Hydroxyzine*	20	18	0		
Lidocaine*	46	44	1		
Codeine*	66	64	2		
Morphine*	81	78	2		
Thioridazine	3	3	0	3	0
Amitriptyline	9	7	0	8	0
Mianserin	17	16	1	18	1
Pitofenone	26	23	1	25	1
Pethidine	34	33	0	34	1
Acebutolol	43	41	0	44	1
Atropine	50	50	1	51	1
Tubocurarine	55	54	2	56	1
Ephedrine	63	61	2	62	1
Mepyramine	68	67	2	69	1
Sulpiride	71	70	2	72	0
Cimetidine	76	72	2	75	2
Terbutaline	84	80	2	83	1
Procaine	88	84	1	87	1

*Reference compound

The MLL values for different two-system combinations are presented in Table 2. The hR_f values for the TLC_2 have been taken from a comprehensive collection of drug R_f values on standardized systems (7). In section A, separate constant SD values for the systems are used. For the RPTLC and the TLC_1, the SD is the mean of the individual SD values of compounds having $hR_f > 40$ (9). For the TLC_2, the SD has been estimated from published data (7,9). In section B, a common constant SD for all three systems is used.

The RPTLC data in Table 1 were obtained using chromatographic plates from single lot. Table 3 shows the lot-to-lot reproducibility of the RPTLC system and the influences from using a correction method (7) for the R_f values.

DISCUSSION

From several potential evaluation methods, the mean list length approach was chosen as the basis of the current evaluation because of its perspicuity and the ease of treating combined data sets. Reference data by the method are available (8,9). The MLL approach is also substance directed and it can be used in computerized substance identification (9,11).

In the MLL method it is assumed that the R_f of a substance varies according to a normal distribution with an SD dependent on the chromatographic system. For every substance in the data set the probability is calculated that other substances are confused with the substance itself. The probabilities are normalized so that the sum of the probabilities becomes 1, and the substances are ranked in decreasing order of probability. The L for a given drug is the number of other drugs, in addition to the drug itself, that would qualify for identification with a certain cumulative probability level. The MLL is obtained by averaging the individual L values. The shorter the MLL, the better the chromatographic system (9).

The TLC_1 system was originally introduced by Stahl for the separation of opium alkaloids (12) but it has been applied to drug screening in the authors' laboratory for several years. The system was chosen for the evaluation because of its apparently favourable compound distribution with fairly good precision and because of its compatibility with HDEHP extracts. The TLC_2 system is a widely used system for comprehensive drug screening (7). Although the compound distribution produced by the TLC_2 is good, the reproducibility of the system is only moderate.

Normal and reversed phase chromatography involve different separation mechanisms, the former relying mainly

on adsorption and the latter mainly on hydrophobic interactions. RPTLC thus gives additional uncorrelative information about the sample. Polar substances may give bad-shaped spots and quaternary ammonium compounds generally do not migrate at all on normal phase systems, whereas they produce well-shaped spots with reasonable R_f values on the RPTLC. RPTLC systems also provide useful information about the hydrophobicity of the analyte, making structure prediction possible (13).

The merits of the TLC_1/RPTLC pair can be seen in Table 1: the MLL for the RPTLC and for the TLC_1 are 9.7 and 10.9 (95% cumulative probability), respectively, whereas the MLL for their combination is 1.8. This value is better than previously reported for TLC/TLC (9) or TLC/RPTLC (8) combinations. A slightly higher value is obtained for the TLC_2/RPTLC (Table 2), and a considerably higher value for the TLC_1/TLC_2.

The reproducibility of silica gel TLC plates is normally good and the variation of R_f values is mainly due to the effects of environmental conditions on the chromatographic process. RPTLC plates, instead, are more irreproducible, the reversed phase material varying from batch to batch. The RPTLC R_f values obtained here with a single lot of plates are very reproducible because of the system's tolerance against changes in atmospheric humidity and tank saturation. However, Table 3 clearly shows the importance of the correction of the R_f values with reference compounds (7): both accuracy and precision of the corrected R_f values are better than those of the uncorrected, the former differing no more than one unit from the values in Table 1.

It is noteworthy that biological dirt has practically no effect on the R_f values on the RPTLC system, whereas it is known that biological matrix affects both precision and accuracy on normal phase TLC systems (14).

ACKNOWLEDGEMENTS

The authors wish to thank Mr Jani Virtanen and Miss Auli Mikkola for their technical assistance.

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