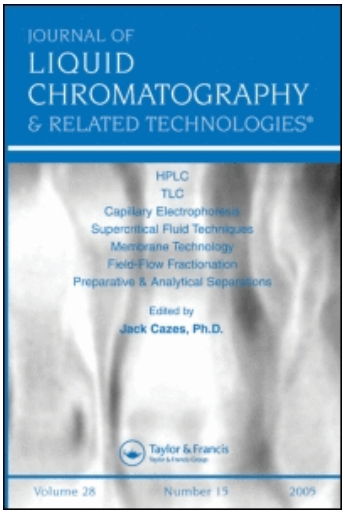

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TOXICOLOGICAL DRUG SCREENING BY OVERPRESSURED LAYER CHROMATOGRAPHY

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ABSTRACT

Two overpressured layer chromatography (OPLC) systems were developed for the screening of toxicologically relevant basic drugs in forensic and clinical contexts. The OPLC1 system was trichloroethylene - methylethylketone - *n*-butanol - acetic acid - water 17+8+25+6+4 and the OPLC2 system was butyl acetate - ethanol - tripropylamine - water 85+9.25+5+0.75 with pre-saturation. Both systems were tested on high performance silica gel plates. The R_f values of the drugs were reproducible, the peak shapes were symmetrical, and the chromatographic systems showed low mutual correlation (r = 0.103). The separation numbers (SN) were 27.5 and 29.2 for OPLC1 and OPLC2, respectively, which are more than two times higher than those obtained with TLC systems in general. The combination of the systems was demonstrated to be feasible in the screening for drugs in autopsy urine samples, utilizing automated identification by hR_f^c/UV library search with combined dual-system reporting.

INTRODUCTION

Despite the many advantages of ordinary free-flow thin-layer chromatography, the relatively low resolving power limits the feasibility of the technique in some application areas, such as broad scale screening analysis. Although this disadvantage can, in part, be compensated by an efficient use of visualization reactions, the full use of *in situ* UV spectral identification is not possible with unresolved fractions. Changes to high-performance TLC plates bring only limited improvement, and this is dependent on the particular chromatographic system used.

There are currently two alternative instrumental means to improve the resolving power at the chromatogram development stage: Automated Multiple Development (AMD)[1-3] and overpressured layer chromatography (OPLC).[4-8] AMD is based on the use of a stepwise gradient from higher to lower elution strengths with increasing elution distances in each partial development. This results in a broad analytical application range and the focusing of the analyte fractions. Separation numbers (SN) of up to forty have been achieved by AMD but several hours' total development times may be necessary. OPLC relies on the forced flow of the mobile phase against an external pressure, which leads to short development times and decreased diffusion of the analyte fractions, making it possible to utilize longer developing distances.

Toxicological drug screening in forensic and clinical context is a field where TLC has already proved to be successful but where improved resolving power would be highly beneficial.[9-12] Considering the time factor, OPLC would appear to be the technique of choice in these applications. Although OPLC has been used frequently in drug analysis, the studies published have focused mainly on the separation of chemically closely related compounds in a certain pharmacological category[13-19] or those compounds originating from a certain botanical source.[20-25] This paper describes two OPLC systems designed for the comprehensive screening of basic drugs and demonstrates their feasibility with authentic toxicological urine samples.

EXPERIMENTAL

Materials and Apparatus

The chromatographic plates were 20 x 20 cm HPTLC aluminium sheets coated with 0.2 mm layers of silica gel 60 F_{254} (Merck, Darmstadt, Germany). The plate edges were factory-sealed for OPLC use (OPLC-NIT Engineering Company, Budapest, Hungary). Drug standards were obtained from various pharmaceutical companies and were of pharmaceutical purity.

Table 1

Corrected R_f Values (hR_f^c) of 82 Toxicologically Important Drugs and Metabolites

Drug	hR_f^c		Drug	hR_f^c	
	OPLC1	OPLC2		OPLC1	OPLC2
Acebutolol	37	3	Moclobemide	36	29
Alprenolol	61	14	Moperone	76*	57
Aminophenazone	55	53	Norchlorprothixene1	58	7
Amitriptyline	45	40*	Norchlorprothixene2	62	13
Atenolol	19	1	Norcitalopram	44	2
Betaxolol	55	11	Norclomipramine	62	7
Bisoprolol	49	9	Norcodeine	29	1
Buspirone	47	68	Nordoxepin	52	5
Caffeine	80	41	Norlevomepromazine	53	9
Carvedilol	80	0	Normianserin	62	18
Celiprolol	39	3	Nororphenadrine	55	13
Chlorcyclizine	47	41	Norpromazine	49	3
Chloroquine	3	3	Nortrimipramine	60	14
Chlorpromazine	45	39	Nortriptyline	58*	8
Chlorprothixene	48	53	Norverapamil	77	27
Citalopram	32	14	Oxprenolol	51	12
Clomipramine	49	42	Paroxetine	62	4
Clozapine	31	27	Pentoxifylline	79	37
Codeine	16*	9*	Periciazine	42	28
Cyclizine	46	47	Perphenazine	32	12
Desipramine	56	4	Phenazone	84	36
Dextromethorphan	43	11	Pindolol	55	11
Dextropropoxyphene	46	94*	Practolol	27	2
Diacetylmorphine	26	21	Prazosin	45	64
Diltiazem	34	34	Prochlorperazine	31	1
Disopyramide	28	20	Promazine	38*	19*
Doxepin	40	30	Promethazine	12	56
Ethylmorphine	22	10	Propranolol	57	12
Flecainide	59	18	Quinidine	59	15
Fluoxetine	57	7	Quinine	58	11
Fluvoxamine	54	4	Ranitidine	5	1
Hydroxychloroquine	2	2	Sotalol	30	4
Hydroxyzine	39	40	Sulpiride	5	3
Imipramine	44	29	Theophylline	86*	19
Ketamine	41	94	Thioridazine	49	36
Labetalol	78	10	Trazodone	45	53
Levomepromazine	41	60*	Trimethoprim	48	13
Lidocaine	28	98	Trimipramine	46	76
Melperone	49	60	Verapamil	50	57
Metoclopramide	16	7	Zopiclone	13	16
Metoprolol	47	9	Zuclopenthixol1	33	12
Mianserin	52	54	Zuclopenthixol2	36	14

*R_f correction standard

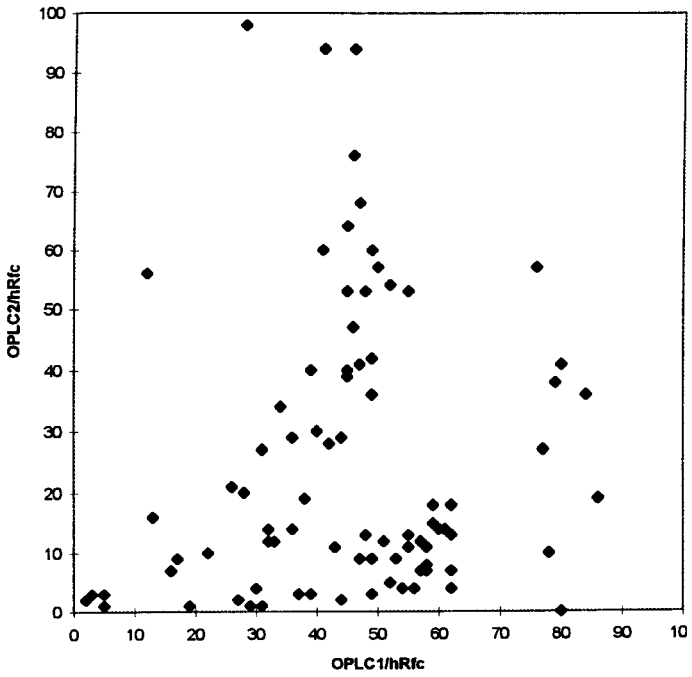


Figure 1. Correlation of hR_f^c values of OPLC1 and OPLC2 systems.

The standard and correction standard solutions were prepared in methanol to obtain a concentration of 2 mg/mL and 1 mg/mL, respectively. Analytical grade solvents were used throughout the study. The urine samples were collected at autopsy.

The automatic TLC sampler was an ATS III (Camag, Muttenz, Switzerland). The OPLC instrument was a Personal OPLC Basic System 50 (OPLC-NIT Engineering Company). The scanning densitometer was a TLC Scanner 3 (Camag) operated with Cats 4.03 software. The Dual Plate Spectrum Library software for Windows (Sunicom, Helsinki, Finland)[12] was utilized.

Chromatography

OPLC1: The mobile phase composition was trichloroethylene – methyl-ethylketone - *n*-butanol - acetic acid - water 17+8+25+6+4, and the development was carried out without the pre-saturation of the plate.

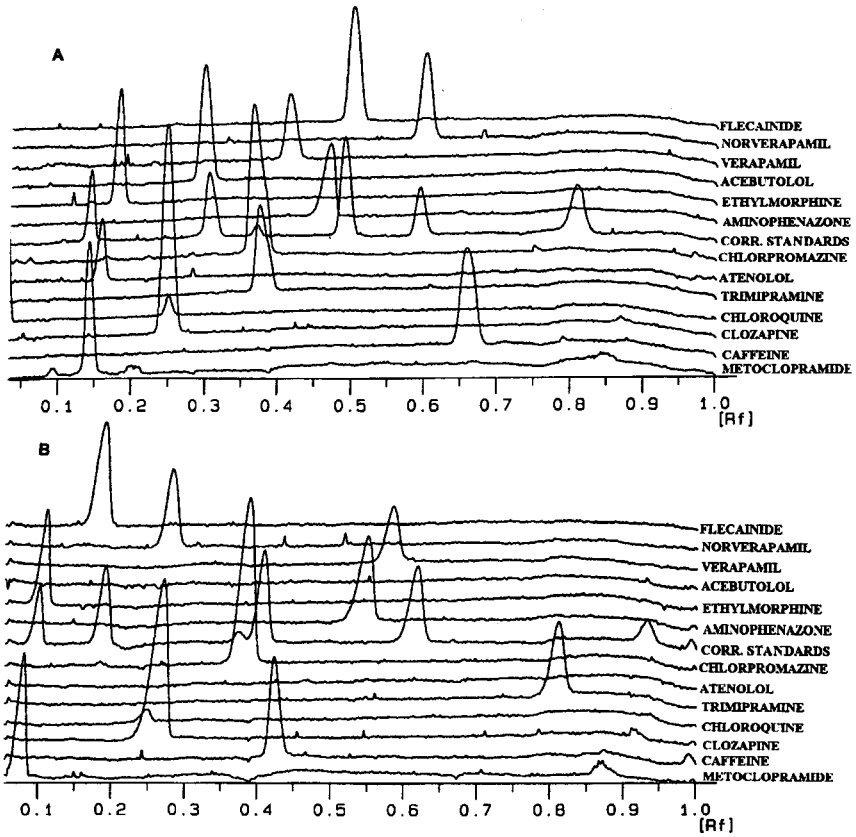


Figure 2. Chromatography of selected basic drugs on (A) OPLC1 and (B) OPLC2.

The external pressure was 50 bar, the flow-rate was 450 µL/min, the volume of rapid delivery was 300 µL, and the mobile phase volume was 5500 µL. The correction standards were codeine (hR_f^c = 16), promazine (hR_f^c = 38), nortriptyline (hR_f^c = 58), moperone (hR_f^c = 76), and theophylline (hR_f^c = 86).

OPLC2: The mobile phase composition was butyl acetate - ethanol (96.1% V/V) - tripropylamine - water 85+9.25+5+0.75, and the plate was saturated with the mobile phase for 0.5 h in a tank with filter paper immediately prior to development. The external pressure was 50 bar, the flow-rate was 450 µL/min, the volume of rapid delivery was 300 µL, and the mobile phase volume was 5000 µL.

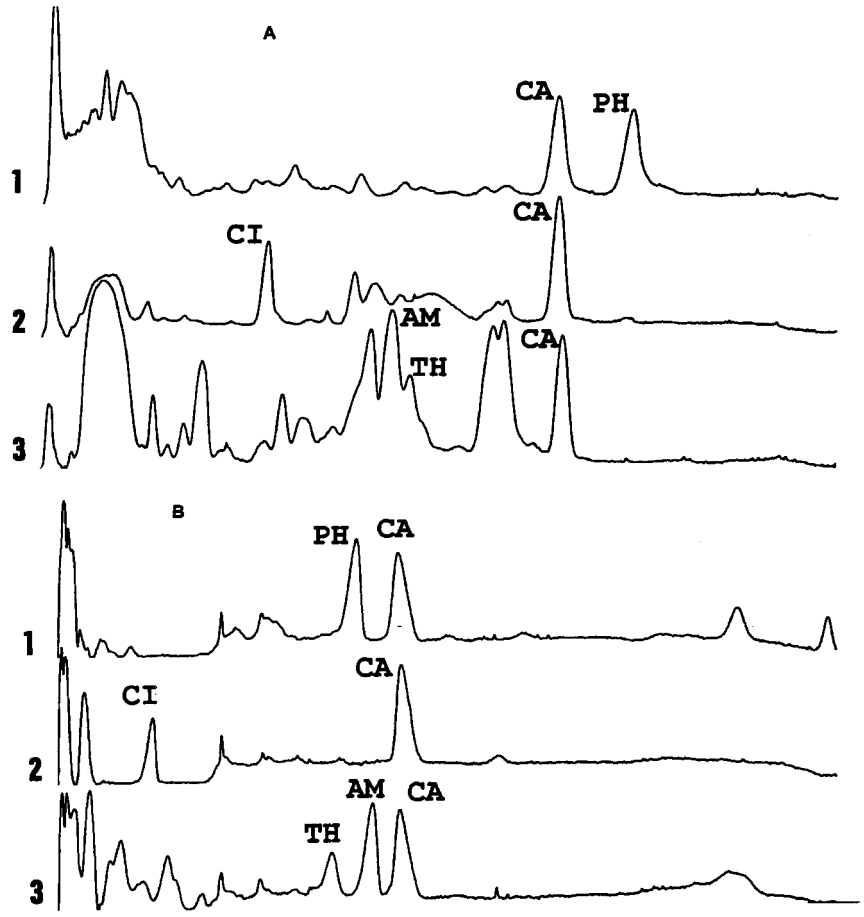


Figure 3. Analysis of urine extracts on (A) OPLC1 and (B) OPLC2. Main findings: CA=Caffeine, PH=Phenazone, CI=Citalopram, AM=Amitriptyline, TH=Thioridazine.

The correction standards were codeine (hR_f^c = 9), promazine (hR_f^c = 19), amitriptyline (hR_f^c = 40), levomepromazine (hR_f^c = 60) and dextropropoxyphene (hR_f^c = 94). The measurement of the corrected R_f values (hR_f^c) listed in Table 1 was carried out by performing three chromatographic runs of the drug standards, together with the correction standard mixture on a separate track, on each system during a two-week period. Standard and correction standard solutions (0.5 µL) were applied band-wise to the plate with the automatic TLC sampler.

Urine Analysis

The sample preparation procedure for basic drugs in 5 mL urine specimens involved an ion-pair extraction with dichloromethane containing 0.01 M bis(2-ethylhexyl)phosphoric acid at pH 7.5.[11] Aliquots of the reconstituted extracts (10 µL) and the correction standard mixture on a separate track were applied band-wise to the plates with the automatic TLC sampler. The developed plates were evaluated by densitometry at 220 nm, and the *in situ* UV spectra were measured using a spectral range of 190-400 nm with 5 nm wavelength increments.[26] The Dual Plate Spectrum Library software[12] was used for the correction of R_f values[9] and for the library search based on hR_f^c values and UV spectra.

RESULTS AND DISCUSSION

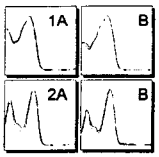
Table 1 shows the corrected R_f values (hR_f^c) of 82 basic drugs and metabolites commonly encountered in forensic and clinical toxicology, analysed on the OPLC1 and OPLC2 systems. The values are the means of three independent developments, with a standard deviation always lower than 2 units. The correlation of the two hR_f^c data sets is 0.103, and the distribution of the values is illustrated graphically in Figure 1. The separation numbers (SN) were 27.5 and 29.2 for OPLC1 and OPLC2, respectively, as measured with 44 randomly selected substances from the present libraries. These values are more than two times higher than those typically obtained with ascending free flow TLC.[27] The developing time was approximately twelve minutes for each OPLC system.

The chromatographic behaviour of a selection of substances on the present systems is shown in Figure 2. The figure demonstrates that the peak shapes are generally symmetrical and no disturbing fronts can be seen over the whole chromatography range.

All the drugs tested possessed migration distances below the apparent R_f = 1 but, due to the nature of the OPLC technique, drugs may exist that elute off the plate on either system.

The analysis of three typical autopsy urine samples is shown in Figure 3. Case 1 involved therapeutic phenazone medication, Case 2 was a citalopram intoxication, and Case 3 was an amitriptyline intoxication while the thioridazine blood levels were therapeutic. In each case, the urine screen by OPLC with hR_f^c/UV library search correctly identified the medication. A dual-plate report for Case 1, combining the findings obtained with both systems,[12] is shown in Figure 4.

No.	Substance Name	Plate	Peak	Position	Diff	Area	Correlation
1.	Phenazone	A	14	82	1	10572.3	0.999021
		B	7	34	1	10989.6	0.998713
2.	Caffeine	A	13	77	2	10488.1	0.993393
		B	8	40	0	10944.7	0.996517



Summary Report - Track: 8

Total Peaks on A: 14
on B: 10

Identified Compounds: 2

Peaks not identified on A: 1 2 3 4 5 6 7 8 9 11 12
on B: 1 2 3 4 5 6 9 10

Figure 4. Dual Plate Spectrum Library identification report for case 1 of Figure 3. The search was performed against an hR_f^c/UV library containing the drugs of Table 1, using a hR_f^c search window of ±3 and a correlation cut-off value of 0.95.

The first OPLC apparatus was constructed as early as twenty years ago but the present instrumentation, being compact and easy to use, for the first time allows the full utilization of the technique in routine analysis. However, method development in OPLC is difficult due to the lack of a gas phase during chromatogram development. The partially wetted zone, caused by the air present in the stationary phase in both the free and adsorbed form, and the secondary fronts obtained with multi-component mobile phases can seriously disturb certain parts of the separation. This is more pronounced in screening analysis, where the whole separation distance should be available. TLC systems, such as those which have become established in the drug screening field,[9] are generally not transferable as such to OPLC, not even with the pre-saturation of the chromatographic plate.

The OPLC1 system is based on the method of Gulyás et al.,[13] which was originally designed for stimulant type doping agents. The system, as such, was tested to be suitable for a broader range of drugs by the present authors but, in the final version, chloroform was replaced by trichloroethylene to obtain more concise analyte fractions. The OPLC2 system was designed to be complementary to the OPLC1 and, thus, a basic mobile phase based on a carbonyl compound was chosen. The amine proved to be the critical component of the mobile phase in order to obtain a method for broad scale drug screening: ammonia and secondary amines could not be used because their strong fronts could not be avoided by adjusting the other components of the mobile phase. Triethylamine was promising but tripropylamine proved to be best, as it produces no front at the critical separation area.

The most concise fractions, in general, were obtained by using a more lipophilic carbonyl compound, butyl acetate, with a small amount of water and by adjusting the final mobile phase strength with an alcohol.

CONCLUSIONS

This study is the first that introduces OPLC methods for comprehensive drug screening, showing that even compounds with large differences in polarity properties can be analyzed by single OPLC methods. However, this requires a very careful adjustment of the mobile phase components to avoid the formation of interfering fronts and to obtain a favorable analyte distribution. The present two methods possess separation numbers over two times higher than ordinary TLC methods and show little mutual correlation.

In addition, the developing times are shorter than generally in TLC. Though the current instrumentation allows easy and well controlled operation, its main disadvantage is the relatively high price of factory-sealed OPLC plates.

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