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MORE ECONOMICAL USE OF HIGH-PERFORMANCE THIN-LAYER PLATES FOR CHROMATOGRAPHIC SCREENING OF ILLICIT DRUG SAMPLES

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SUMMARY

A rapid thin-layer chromatographic method for the separation of illicit drugs and similar compounds by two simultaneous but different developments on the same chromatographic plate is described. One half of the plate was impregnated with an aqueous solution of $KHSO_4$ and the whole plate developed in a Camag linear developing chamber from two opposite sides using methanol with 0.01 *M* KBr as eluent.

INTRODUCTION

Thin-layer chromatography (TLC) is often the preferred method for initial screening of illicit drug samples. Although TLC is a rapid technique (high sample throughput) it is essentially a manual method. Therefore, when TLC is used in combination with automated chromatographic methods [high-performance liquid chromatography (HPLC) and gas–liquid chromatography (GLC)] the time factor for the separation becomes important. High-performance TLC (HPTLC) plates with small particle size and size distribution have been designed for fast separations, but are relatively expensive for large scale analyses. However, when such plates are developed horizontally, *e.g.*, in the Camag linear developing chamber, sample plots can be applied on two opposite sides and as many as 30 samples (10 × 10 cm plates) can be chromatographed in one run. In forensic drug analysis, however, where the number of items in each case examined is usually low (1–5), so many analyses are difficult to administer and the risk of making mistakes increases.

One way to utilize more economically the advantages of HPTLC plates in horizontal development is to perform simultaneously two different separations (from opposite sides) on the same plate. Then, either the solid phase or the eluent (or both) have to be different. It also becomes necessary for the development times for both halves of the plate to be approximately equal for the particular solid–liquid phase combination chosen. In horizontal TLC interferences in the gas phase are likely to

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occur when different solvent systems are used simultaneously. A more attractive approach would therefore be chemically to alter the properties of the thin layer itself. Methods of derivatization of the silica gel are available[1–3] but are generally too complicated and time-consuming for a screening method. A convenient way to modify the adsorbent is to use different kinds of impregnation agents[1]. Salts of metal ions have been employed to modify separations of alkaloids on silica gel and alumina[4]. Systems of high discriminating power for basic drugs were obtained by dipping plates into solutions of different pH values[5] or by addition of ion-pair forming salts[6,7].

This report describes a method which combines the advantages of changing the protolytic properties of the adsorbent and the addition of salts to the eluent for two simultaneous but different separations of drugs on the same TLC plate.

EXPERIMENTAL

All chemicals were of pharmaceutical grade and of different origins. The analytical grade methanol eluent (E. Merck, Darmstadt, G.F.R.) was used without prior purification.

Preparation of plates

The adsorbent layer of the plates (Merck pre-coated HPTLC plates, silica gel G 60 F_{254}, 10 × 10 cm) was divided in two equal halves by scoring with a thick needle. Then, one half of the plate was immersed in aqueous $KHSO_4$ (0.1 *M*) for *ca.* 10 sec, air-dried at room temperature for half an hour and then at 120°C for 1 h.

Thin-layer chromatography

0.5-μl Standard solutions (10 mg/ml in 80% ethanol) were applied to the plates using 1-μl glass capillaries (Microcaps; Drummond, PA, U.S.A.). Two opposite sides (of different pH) were spotted 3 mm from the bottom edges of the plate. Spot size: diameter ≤1 mm. A Camag 10 × 10 cm linear developing chamber (Camag, Muttenz, Switzerland) was used. Each trough was filled with the developing solvent (0.0125 *M* solution of KBr in methanol, 2 × 1.0 ml) and a 2-mm counter plate was used.

Detection

Developed chromatograms were visually examined under a UV-lamp at 254 and 332 nm, then sprayed with a modified solution of ninhydrin (0.3 g ninhydrin, 2 ml glacial acetic acid, 2 g sodium acetate, 5 ml water, made up with ethanol to 100 ml) and placed in an oven at 130°C for 5–10 min. For trace analyses, plates were allowed to stand in the oven for 20 min. After cooling, the plates were sprayed with iodoplatinate[10].

RESULTS AND DISCUSSION

When selecting suitable conditions for the simultaneous analysis on the same chromatographic plate, systems of low correlation are desirable. A number of such systems have been reported[5,6] but their use is not always compatible with a linear developing chamber. Single solvent systems of comparatively high polarity could be

TABLE I

hR_F VALUES OF ILLICIT DRUGS AND SIMILAR COMPOUNDS IN DIFFERENT TLC SYSTEMS

No.	*Substance*	*Untreated plate, c_{KBr} (M)*			*Acidified plate, c_{KBr} (M)*			pK_{HA}-*	*Amine type***
		0	*0.01*	*0.10*	*0*	*0.01*	*0.10*		
1	Amfepramone	70	71	74	28	54	74		
2	Amitriptyline	33	35	52	30	43	59	9.4	3° ali
3	Amphetamine	13	30	72	53	66	85	9.9	1° ali
4	Benzocaine	91	93	94	91	93	96	2.5	1° arom
5	Bromo-STP	7	26	82	51	67	92		1° ali
6	Caffeine	70	70	72	69	69	67	1.2	3° conj
7	Cocaine	46	49	54	18	45	55	8.6	3° ali, cy
8	Codeine	20	21	32	12	21	53	8.2	3° ali, cy
9	Dextropropoxyphene	60	60	70	37	59	84		3° ali
10	Diazepam	87	93	92	91	91	88		3° conj
11	Ephedrine	7	21	67	49	61	89	9.6	2° ali
12	N-Ethylamphetamine	16	33	78	53	68	83		2° ali
13	N-Ethylmorphine	21	24	38	14	30	51	8.1	3° ali, cy
14	Flurazepam	67	67	68	25	45	64		3° ali
15	Heroin	32	34	36	13	23	53	7.6	3° ali, cy
16	*p*-Hydroxyamphetamine	8	27	82	41	79	91	9.3	1° ali
17	Levomethorphan	9	14	43	24	40	63		3° ali
18	Lidocaine	82	84	85	31	54	75	7.8	3° ali
19	Mescaline	5	16	47	22	52	82		1° ali
20	Methadone	20	33	65	37	61	81	8.9	3° ali
21	Methaqualone	89	90	93	88	90	91		3° conj
22	N-Methylamphetamine	10	24	54	38	59	79	10.1	2° ali
23	Morphine	22	24	37	13	26	56	8.2	3° ali, cy
24	Narcotine	84	83	83	32	40	65	6.4	3° ali, cy
25	Nicotine	56	56	52	7	24	37	8.0	3° ali, cy
26	Nitrazepam	92	93	93	90	94	93		3° conj
27	Norephedrine	15	30	75	67	68	93		1° ali
28	Nortriptyline	11	35	49	42	60	68	10.0	2° ali
29	Oxazepam	90	94	95	91	94	95		3° conj
30	Papaverine	86	84	86	39	51	70	6.4	3° conj
31	Paracetamol	92	92	93	93	92	93		
32	Phenacetine	91	90	95	91	93	93		
33	Phenazone	79	81	83	78	79	78		3° conj
34	Phencyclidine	3	9	41	15	35	61		3° ali, cy
35	Phendimetrazine	61	60	63	15	38	55	7.6	3° ali, cy
36	Phenmetrazine	41	45	52	33	51	74	8.4	2° ali, cy
37	Procaine	51	53	59	27	49	67	9.0	3° ali
38	Propylhexedrine	7	25	66	46	63	87		2° ali
39	Pyridostigmine	2	11	38	9	24	41		4° conj
40	Quinine	35	35	56	36	45	76	8.5	3° ali, cy
41	Synstigmine	2	9	65	10	24	83		4° arom
42	Tebaine	25	27	40	12	30	54	8.2	3° ali, cy
43	Tetracaine	47	50	53	19	39	68	8.5	3° ali

* For dibasic amines, values for the first dissociation step only are tabulated. Data from refs. 8, 9.

** For the most basic nitrogen. Abbreviations: ali = aliphatic; arom = aromatic; cy = cyclic; conj = conjugated; 1° = primary; 2° = secondary; 3° = tertiary and 4° = quaternary.

used in combination, *e.g.*, acetone, methanol, acetonitrile and tetrahydrofuran, and little influence of vapour mixing on R_F values was observed. However, such combinations are usually too similar in polarity to allow useful discrimination of drugs. With binary and tertiary eluents, gas-phase interactions became important and in some cases solvent demixing occurred. As expected, the risk of solvent demixing is more pronounced in horizontal development where no pre-saturation of the eluent is possible.

More useful results were obtained by changing the pH value of the silica layer. Acidification was accomplished by dipping one half of the plate in aqueous $KHSO_4$. The low solubility of $KHSO_4$ in methanol reduced the wash-out effects noted by others[6] for phosphate buffer.

Chromatographic data for illicit drugs and similar compounds on untreated and acidified silica gel using methanol as the eluent are shown in Table I. It can be seen that several compounds change their order of elution on going from untreated to acidified silica. Since both systems showed some tailing, particularly on the acidic layer, KBr was added to the eluent. In a similar study, De Zeeuw *et al.*[6] noted improved chromatographic behaviour with methanol systems on addition of bromide and chloride at concentrations of at least 0.1 *M*. In this study, lower concentrations were found appropriate and even at a concentration of 0.01 *M* KBr a marked increase in R_F values, particularly for the most basic compounds, could be observed (*cf.*, Table I). For comparison purposes, data from separations with 0.1 *M* KBr are included. Correlations of data for an eluent concentration of 0.01 *M* KBr are depicted in Fig. 1.

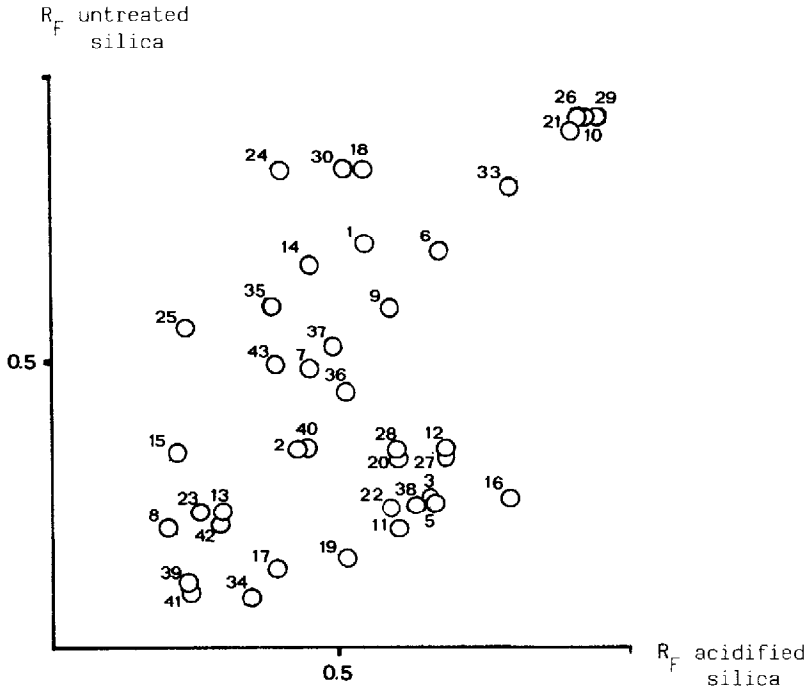

Fig. 1. Correlation of R_F values in systems with 0.01 *M* KBr in methanol as eluent. Numbers refer to substances in Table I.

The correlation factor was calculated to be 0.63 and the discriminating power 0.94, using error factors of 0.05 and 0.07 for untreated and acidified silica respectively[5].

For each substance chromatographed in this way, two R_F values, different UV responses and a number of colour reactions (in this case with ninhydrin and iodoplatinate) can be registered on the same plate. The total elution time is about 11 min and the process is automatically stopped when the fronts reach the scored central line. Highly accurate R_F values can be obtained with HPTLC plates, particularly when small starting spots are applied[11]. By dissolving the samples in a solvent with low velocity coefficient, K (80% ethanol), spots with a diameter of 1 mm or less can be achieved manually. With solvents of higher coefficients (acetone, chloroform) an applicator is recommended. To minimize the risk of concentration effects (from contamination of KBr from previous runs) on retention times, the amount of eluent was carefully adjusted such that all the solvent was used up when the process stopped. With methanol a volume of 1.0 ml in each trough was found to be suitable. Also, the glass strips supplying the plate with eluent should be rinsed after each run to remove any precipitated KBr. Standard deviations for a selected number of compounds obtained by ten different analysts on different occasions are shown in Table II.

One advantage of using shorter development paths is an increased sensitivity of detection of the spots. With ninhydrin, 100 ng of amphetamine could be detected on the untreated silica (after 20 min at 130°C), compared to 1–5 μg reported on normal TLC plates[12]. The reaction of ninhydrin with amines and similar compounds has been studied in some detail and different mechanisms have been suggested to explain the results[12,13]. A considerable increase in sensitivity of detection of amphetamine from 5 to 1 μg on the acidic layer could be obtained if the ninhydrin spray was modified by the addition of 2% acetic acid–2% sodium acetate. These results might suggest an acid–base catalytic effect. The somewhat higher limit of detection on the acidic side compared to the untreated is mainly due to elongated spots obtained for the most basic substances.

It is clear from inspection of the data in Table I that basic and quaternary

TABLE II

STANDARD DEVIATIONS FOR SELECTED COMPOUNDS

Data obtained by ten different persons on different occasions.

Compound	*Untreated silica*		*Acidified silica*	
	hR_F	*S.D.*	hR_F	*S.D.*
Amphetamine	30	2.35	66	3.34
Caffeine	70	1.79	69	1.24
Cocaine	49	2.67	45	3.27
Codeine	21	1.23	21	1.06
Diazepam	92	1.42	91	1.48
Heroin	34	0.99	23	1.79
N-Methylamphetamine	24	2.33	59	3.04
Phenazone	81	0.75	79	1.23
Phenmetrazine	45	1.84	51	3.81
Procaine	53	2.13	49	1.85

compounds are less strongly retarded with increasing concentrations of bromide in the methanol eluent. Similar observations were also made from experiments with added chloride and nitrate and, contradictory to results from ordinary TLC plates[6], the R_F values were of the same magnitude. Both ion-pair formation and ion-exchange mechanisms[6,14,15] have been suggested to explain the behaviour of basic drugs on silica using non-aqueous eluents. In both mechanisms, the pH value of the system and the protolytic properties of the solutes are of major importance. This is clearly demonstrated from the data presented in Table I. All compounds studied can be nicely arranged in four groups according to their retention characteristics in the different systems.

Group I: Higher R_F values observed on the acidic half than on untreated half of the plate at zero concentration of KBr. Increased R_F values on both halves on increasing the concentration of KBr in the eluent.

Group II: Lower R_F values on the acidic half than on the untreated half of the plate at zero concentration of KBr. Increased R_F values on both halves on increasing the concentration of KBr.

Group III: Lower R_F values on the acidic half than on the untreated half of the plate at zero concentration of KBr. Increased R_F values only on the acidic half on increasing the KBr concentration.

Group IV: Same R_F value on both halves of the plate independently of pH and concentration of KBr in the eluent.

To Group I belong primary, secondary and tertiary aliphatic amines of high basicity ($pK_{HA} > 9$). Typical examples are the amphetamines and mescaline. Included here also are quaternary compounds. Two exceptions, phencyclidine and levomethorphan, which are cyclic amines, are noted. Members of Group II have somewhat lower pK_{HA} values, ranging from 8.1 (tebaine) to 8.6 (cocaine). They are typically secondary and tertiary, saturated, cyclic amines, *e.g.*, the morphines. Exceptions are tetracaine and procaine (pK_{HA} 9), which are open chain, aliphatic amines. Group III consists mainly of tertiary aliphatic amines (both straight chain and cyclic) having pK_{HA} values ranging from 6.2 (narcotine) to 8.0 (nicotine). One exception is papaverine, which is a conjugated amine. Group IV comprises very weak basis, *e.g.*, caffeine, diazepam, which are usually conjugated amines. Included in this group also are neutral (phenacetine) and acidic (paracetamol) compounds. Although there is some overlap between the groups (particularly between Groups II and III), the findings imply that TLC data can be of some value for obtaining structural information.

In conclusion, a convenient, fast and reliable HPTLC method has been developed which permits two different analyses of basic drugs and similar compounds to be performed simultaneously on the same plate. Very small volumes of eluent are needed. Also, methanol is a solvent of comparatively low inhalational toxicity. One disadvantage of the method is that compounds of similar structure and basicity, *e.g.*, amphetamine and ephedrine, are less well resolved. Therefore, in forensic applications the method should be used in combination with other chromatographic techniques, such as HPLC[14] and GLC[16].

REFERENCES

1 H. J. Issaq, *J. Liquid Chromatogr.*, 3 (1980) 1423.

2 M. Okamoto and F. Yamada, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 163.
3 T. Omori, M. Okamoto and F. Yamada, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 47.
4 N. V. Rama Rao and S. N. Tandon, *J. Chromatogr. Sci.*, 16 (1978) 158.
5 A. C. Moffat and K. W. Smalldon, *J. Chromatogr.*, 90 (1974) 9.
6 R. A. de Zeeuw, F. J. W. van Mansvelt and J. E. Greving, *J. Chromatogr.*, 148 (1978) 255.
7 H. J. Huizing and T. M. Malingré, *J. Chromatogr.*, 205 (1981) 218.
8 D. D. Perrin (Editor), *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965, and Supplement, 1972.
9 G. Schill, *Acta Pharm. Suecica*, 2 (1965) 13.
10 E. G. C. Clarke (Editor), *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969.
11 R. E. Kaiser, in A. Zlatkis and R. E. Kaiser (Editors), *HPTLC —High Performance Thin-Layer Chromatography (Journal of Chromatography Library*, Vol. 9), Elsevier, Amsterdam, Institute of Chromatography, Bad Dürkheim, 1977, p. 85.
12 P. J. Cashman, J. D. Beede and J. I. Thornton, *J. Forensic Sci. Soc.*, 19 (1979) 137.
13 A. Schonberg and E. Singer, *Tetrahedron Report*, No. 51, Pergamon, Oxford, 1978.
14 B. B. Wheals, *J. Chromatogr.*, 187 (1980) 65.
15 R. J. Flanagan, G. C. A. Storey, R. K. Bhamra and I. Jane, *J. Chromatogr.*, 247 (1982) 15.
16 S. Alm, S. Jonson, H. Karlsson and E. G. Sundholm, *J. Chromatogr.*, 254 (1983) 179.