Some chromatographic and electrophoretic data for amphetamine-like drugs

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Numerous thin-layer chromatographic methods have been described recently for the determination of drugs of abuse in blood and urine[1–6]. Usually one single solvent system and one thin layer are used and most of the methods refer to only a few of the amphetamine-type drugs.

We felt that there was still room for improvement of these methods, especially concerning the establishment of the identity of the drug detected. For this purpose, we have examined a wide range of amphetamine-like drugs and have used a wider range of flat-bed methods than previously reported.

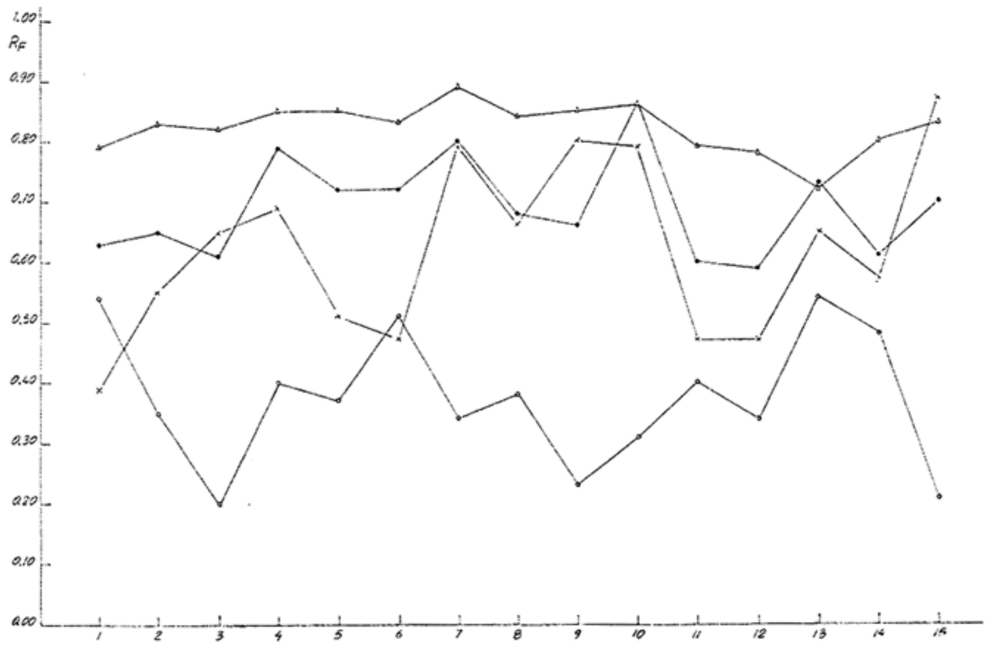

Fig. 1. R_F values of amphetamines on various thin layers with *n*-butanol–formic acid–water (20:1:2) as solvent. The numbers of the compounds correspond to those in Table I. ●, Cellulose "Merck"; ×, alumina "Baker-flex"; ○, silica gel "Merck"; △, polyamide "Baker-flex".

Thin-layer chromatography on a range of supports

In a recent note[7] we pointed out the usefulness of using a single (suitable) solvent on a range of ready thin layers.

Fig. 1 and Table I show the results obtained with butanol–formic acid–water (20:1:2) and cellulose, silica gel, polyamide and alumina layers. Clearly, a much better characterisation is possible than with a single system.

Thin-layer chromatography with several solvents and two-dimensional development

Table II and Fig. 2 give the R_F values for one acidic and two ammoniacal solvents on silica gel plates, and here again good differentiation of the compounds examined can be obtained.

TABLE I

R_F VALUES OF AMPHETAMINES ON VARIOUS THIN LAYERS WITH *n*-BUTANOL–FORMIC ACID–WATER (20:1:2) as solvent

Detection reagents: (a) bromocresol green, 0.5% in ethanol–phosphate buffer of pH 5.5–water (2:1:1). (b) 0.5% Fast blue in water, (c) 1 g of cobalt chloride and 3 g of ammonium thiocyanate in 20 ml of water. (d) Iodine vapour.

No.	*Compounds*	*DC Fertigplatten Zellulose "Merck" (ohne Fluoreszenz-indikator)*	*DC Fertigplatten Kieselgel "Merck" (ohne Fluoreszenz-indikator)*	*Aluminium Oxide IB-F Baker-flex*	*Polyamide 6 Baker-flex*
	Detecting reagent	*a*	*b*	*c*	*d*
1	Amphetamine	0.63	0.54	0.39	0.79
2	Methamphetamine	0.65	0.35	0.55	0.83
3	Dimethamphetamine	0.61	0.20	0.65	0.82
4	Ethylamphetamine	0.79	0.40	0.69	0.85
5	Methoxyphenamine	0.72	0.37	0.51	0.85
6	Phentermine	0.72	0.51	0.47	0.83
7	Methylphenidate	0.80	0.34	0.79	0.89
8	Phenmetrazine	0.68	0.38	0.66	0.84
9	Mephentermine	0.66	0.23	0.80	0.85
10	Prolintane	0.86	0.31	0.79	0.86
11	Ephedrine	0.60	0.40	0.47	0.79
12	Pseudoephedrine	0.59	0.34	0.47	0.78
13	Phencamphamine	0.73	0.54	0.65	0.72
14	Tranylcypromine	0.61	0.53	0.57	0.80
15	Diethylpropione	0.70	0.21	0.87	0.83

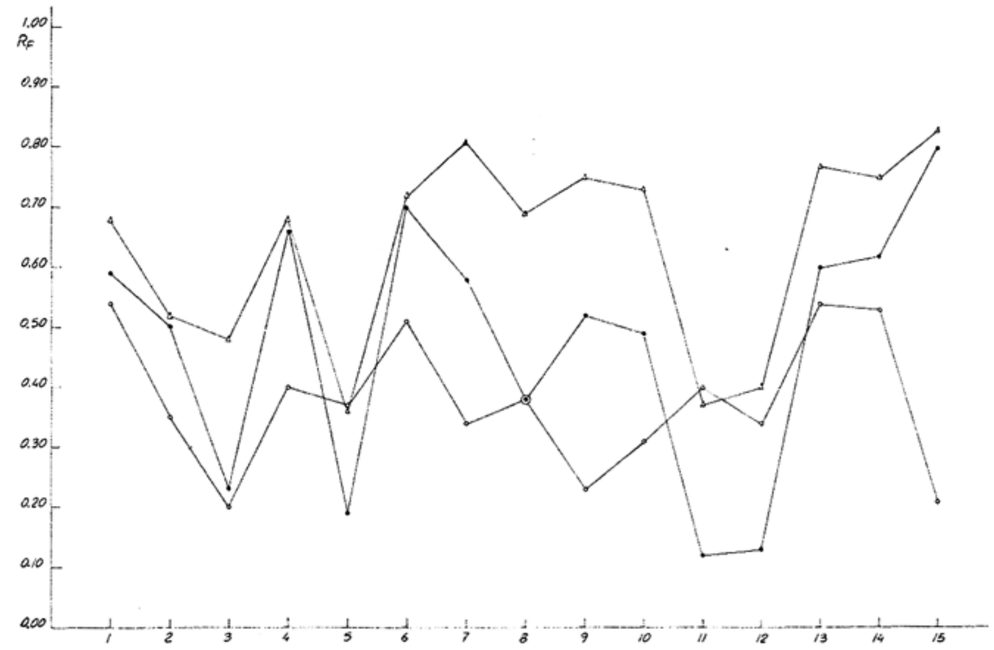

Fig. 2. R_F values of amphetamines on silica gel (DC Fertigplatten Kieselgel "Merck") with three different solvents. The numbers of the compounds correspond to those in Tables I and II. Solvents: ○, *n*-butanol–formic acid–water (20:1:2); ●, *n*-butanol–5 *N* ammonia (1:1); △, ethanol–5 *N* ammonia (9:1).

TABLE II

R_F VALUES OF AMPHETAMINES ON DC FERTIGPLATTEN KIESELGEL "MERCK" (OHNE FLUORESZENZ-INDIKATOR) WITH SOME ALKALINE SOLVENTS

Detection reagent: 0.5% Fast blue in water.

No.	Compound	*Ethanol–5 N ammonia (9:1)*	*n-Amyl alcohol–5 N ammonia (1:1)*
1	Amphetamine	0.68	0.59
2	Methamphetamine	0.52	0.50
3	Dimethamphetamine	0.48	0.23
4	Ethylamphetamine	0.68	0.66
5	Methoxyphenamine	0.36	0.19
6	Phentermine	0.72	0.70
7	Methylphenidate	0.81	0.58
8	Phenmetrazine	0.69	0.38
9	Mephentermine	0.75	0.52
10	Prolintane	0.73	0.49
11	Ephedrine	0.37	0.12
12	Pseudoephedrine	0.40	0.13
13	Phencamphamine	0.77	0.60
14	Tranylcypromine	0.75	0.62
15	Diethylpropione	0.83	0.80

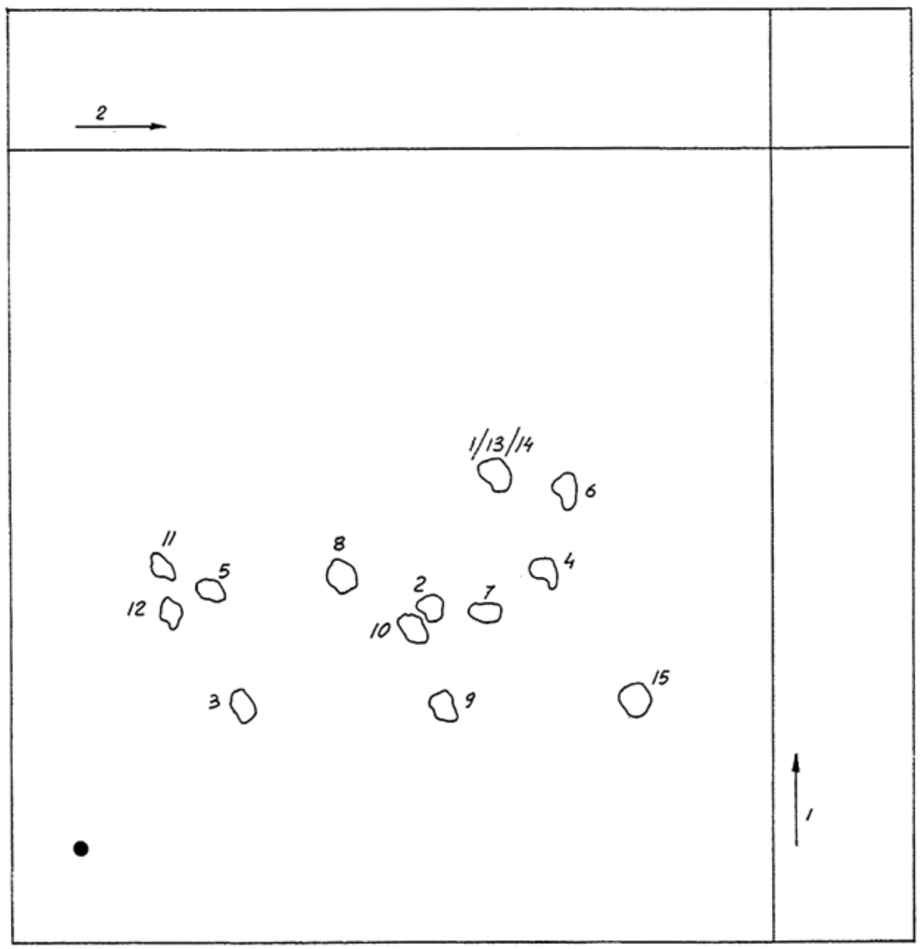

Fig. 3. A two-dimensional chromatogram of an artificial mixture of fifteen amphetamines on a silica gel plate (DC Fertigplatte Kieselgel "Merck"). Solvent 1: *n*-butanol–formic acid–water (20:1:2). Solvent 2: *n*-amyl alcohol–5 *N* ammonia (1:1).

TABLE III

ELECTROPHORETIC MOVEMENT (in mm) OF AMPHETAMINES

Apparatus: Camag high-voltage paper electrophoresis apparatus. Paper: Whatman No. I. Voltage: 2000 V. Time: 30 min. Temperature of circulating water: 7–8°.
Detection reagent: 0.5% Fast blue in water or Dragendorff reagent.

No.	Compound	*0.1 N Acetic acid, pH 2.37*	*0.1 N Acetic acid–0.1 N sodium acetate (1:1), pH 4.61*	*0.5 N Acetic acid–0.5 N sodium acetate (1:1), pH 4.60*	*1 N Acetic acid–1 N sodium acetate, (1:1), pH 4.63*	*Phosphate buffer of pH 7.15, KH_2PO_4 0.03 M–Na_2HPO_4 0.076 M*	*Phosphate buffer of pH 8.10, KH_2PO_4 0.004 M–Na_2HPO_4 0.083 M*
1	Amphetamine	63	74	81	75	58	69
2	Methamphetamine	63	71	79	73	57	70
3	Dimethamphetamine	69	68	79	73	57	69
4	Ethylamphetamine	68	66	65	61	57	69
5	Methoxyphenamine	63	62	70	61	53	67
6	Phentermine	63	67	73	61	54	66
7	Methylphenidate	59	57	57	53	46	55
8	Phenmetrazine	68	66	71	62	41	43
9	Mephentermine	69	66	70	62	32	26
10	Prolintane	56	54	57	51	49	59
11	Ephedrine	63	68	73	67	50	62
12	Pseudoephedrine	63	68	73	67	50	62
13	Phencanphamine	51	50	55	50	46	58
14	Tranylcypromine	74	75	82	75	54	60
15	Diethylpropione	55	55	65	63	54	58

Fig. 3 shows a two-dimensional chromatogram actually obtained with artificial mixtures prepared from samples of pure drugs.

Paper electrophoresis

Using a CAMAG high-voltage paper electrophoresis apparatus, the results shown in Table III were obtained.

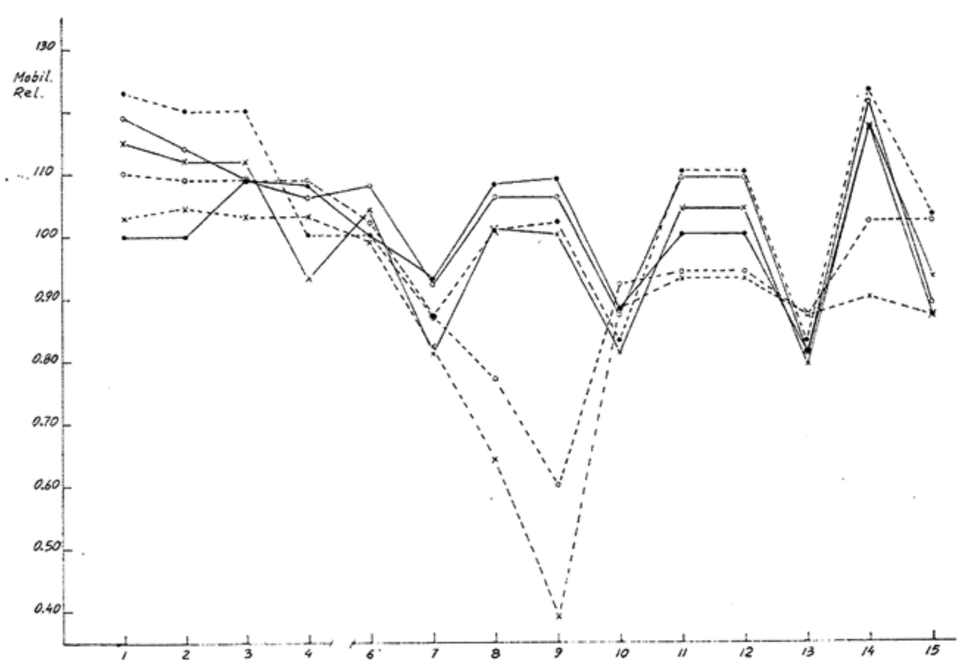

Fig. 4. Electrophoretic movement relative to methoxyphenamine of amphetamines in several buffers. Conditions and compound numbers correspond to those in Table III. ●—, 0.1 *N* acetic acid, pH 2.37, $\mu = 5 \cdot 10^{-3}$ *M*; ○—, 0.1 *N* acetic acid–0.1 *N* sodium acetate (1:1), pH 4.61, $\mu = 5 \cdot 10^{-2}$ *M*; ×—, 0.5 *N* acetic acid–0.5 *N* sodium acetate (1:1), pH 4.60, $\mu = 2.5 \cdot 10^{-1}$ *M*; ●-----, 1 *N* acetic acid–1 *N* sodium acetate (1:1), pH 4.63, $\mu = 5 \cdot 10^{-1}$ *M*; ○-----, 0.03 *M* KH_2PO_4–0.076 *M* Na_2HPO_4; pH 7.15, $\mu = 2.5 \cdot 10^{-1}$ *M*; ×-----, 0.004 *M* KH_2PO_4–0.083 *M* Na_2HPO_4, pH 8.10, $\mu = 10^{-1}$ *M*.

TABLE IV

R_F VALUES OF THE DANSYL DERIVATIVES OF AMPHETAMINES

Spots detected under UV light at 366 nm.

Compound	*Sorbent*	*Kieselgel "Merck"*	*Kieselgel "Merck"*	*Kieselgel "Merck"*	*Alumina IB-F Baker-flex*
	Solvent system	*Ethyl acetate–cyclohexane (75:50)*	*Benzene–triethylamine (100:20)*	*Benzene–cyclohexane–methanol (80:10:2)*	*Benzene–cyclohexane–methanol (80:10:2)*
Amphetamine		0.49	0.38	0.46	0.69
Methamphetamine		0.50	0.52	0.77	0.92
Phenmetrazine		0.50	0.52	0.71	0.92
Ephedrine		0.46	0.44	0.30	0.53
Pseudoephedrine		0.41	0.42	0.31	0.53
Tranylcypromine		0.48	0.35	0.42	0.60

Some good results can be obtained at pH 7.15 and 8.1, but the results show that with these compounds paper electrophoresis is much less specific than thin-layer chromatography.

Dansyl derivatives and their separation by thin-layer chromatography

Dansyl derivatives were prepared as follows: 100 μg of the amphetamine drug were dissolved in 0.1 ml of diethyl ether and mixed with 0.2 ml of 0.1 *N* HCl and the ether was driven off in a current of warm air. Then 0.1 ml of a 0.2 % solution of dansyl chloride in acetone was added and the mixture neutralised with a 1 % solution of sodium bicarbonate and left at room temperature overnight.

The dansyl derivative (together with unreacted dansyl chloride) was then extracted with three 1-ml portions of ethyl acetate, and the extracts were combined and evaporated to a volume of 1 ml.

Only a few of the amphetamine drugs yield dansyl derivatives and their R_F values in various systems are shown in Table IV. The spots are visible under UV light at 366 nm and as little as 0.03 μg of the amphetamines could be detected.

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1 G. P. CARTONI AND A. CAVALLI, *J. Chromatogr.*, 37 (1968) 158.
2 H. ALFER AND D. CLASING, *Deut. Z. Gesamte Gerichtl. Med.*, 64 (1969) 235.
3 J. BROICH, D. B. HOFFMAN, S. ANDRYAUSKAS, L. GALANTE AND C. J. UMBERGER, *J. Chromatogr.*, 60 (1971) 95.
4 K. K. KAISTHA AND J. H. JAFFE, *J. Chromatogr.*, 60 (1971) 83.
5 J. K. BROWN, L. SHAPAZIAN AND G. D. GRIFFIN, *J. Chromatogr.*, 64 (1972) 129.
6 S. J. MULÉ, M. L. BASTOS, D. JUKOFSKY AND E. SAFFER, *J. Chromatogr.*, 63 (1971) 289.
7 I. SIMON AND M. LEDERER, *J. Chromatogr.*, 63 (1971) 448.

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