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QUALITATIVE ORGANIC ANALYSIS

I. IDENTIFICATION OF DRUGS BY PRINCIPAL COMPONENTS ANALYSIS OF STANDARDIZED THIN-LAYER CHROMATOGRAPHIC DATA IN FOUR ELUENT SYSTEMS

GIUSEPPE MUSUMARRA*, GIUSEPPE SCARLATA and GIUSEPPE CIRMA
Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania (Italy)
GUIDO ROMANO and SILVANA PALAZZO
Istituto di Medicina Legale e delle Assicurazioni, Università di Catania, Via Biblioteca 4, 95124 Catania (Italy)
and
SERGIO CLEMENTI and GIANFRANCO GIULIETTI
Dipartimento di Chimica, Università di Perugia, Via Elce di Sotto 10, 06100 Perugia (Italy)
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SUMMARY

Principal component analysis of standardized R_F values in four eluent systems [ethyl acetate–methanol–30% ammonia (85:10:15), cyclohexane–toluene–diethylamine (65:25:10), ethyl acetate–chloroform (50:50) and acetone, with the plate dipped in potassium hydroxide solution] provided a two-component model which accounts for 73% of the total variance. The "scores" plot allowed the restriction of the range of inquiry to a few candidates. This result is of great practical significance in analytical toxicology, especially when account is taken of the cost, the time, the analytical instrumentation and the simplicity of the calculations required by the method.

INTRODUCTION

The idea of using thin-layer chromatography (TLC) in qualitative organic analysis has long been pursued due to the simplicity, the low cost, the rapidity and the sensitivity of this analytical technique. Obviously a single retention factor, R_F, is not sufficient for the identification of any organic compound and it is evident that more measurements are needed. The R_F values in different eluent systems reported either in graphical representations such as the "chromatographic spectrum"[1] and the "chromatographic profile"[2], or in tables[3] have been considered to be suitable for identification purposes. In this regard the choice of the minumum number of eluent systems containing different information is of crucial importance for the identification of unknowns and has been the topic of several statistical studies. The individual information provided by each eluent system and the correlation between such systems

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have been investigated using the "discrimination power"[3–6], while information theory[7] and numerical taxonomy techniques[8,9] have been used to evaluate the quality of TLC separations and for the selection of optimum sets of eluents.

In this context principal components analysis (PCA) has been proven to have great potential for the identification of basic drugs such as benzodiazepines, phenothiazines and opiates from their R_F values in eight eluents[10] and for the evaluation and the selection of eluent systems in TLC[11,12]. PCA has significant advantages over statistical methods based on the information provided by single systems due to the fact that it enables a direct measure of the properties of each system in combination with the others, and indicates both the minimum set of eluents needed and reliable statistical criteria for their selection. Following these criteria we have recently proposed a minimum set of four eluents containing virtually all the information obtainable from a larger set of 40 eluent mixtures[12]. As a consequence of these results we here report the PCA of the R_F values of 362 drugs in the above set of four eluents with the purpose of achieving a drastic restriction in the range of inquiry and hopefully identification of unknown samples. The examined compounds (basic, neutral and a few acidic drugs), which include substances widely used in Italy for therapeutic purposes and well known drugs of abuse, are nitrogen bases which can be detected using the Dragendorff reagent and acidified iodoplatinate solution[13].

EXPERIMENTAL

The drugs are named according to the Merck Index[14]; for substances not reported therein, either the nomenclature adopted by Clarke[13] or that of Chemical Abstracts is used.

R_F Measurements

The eluent compositions are reported in Table I, together with the $R_F \times 100$ values for four reference compounds in each system. These $R_F \times 100$ values were used to correct the experimentally determined $R_F \times 100$ values (see below). The corrected $R_Fc \times 100$ values for compounds 1–362 in eluent mixtures I–IV are reported in Table II.

The drug (10 mg) was dissolved as the free base form or as the hydrochloride salt (different salts are explicitly stated in Table II) in methanol (5 ml), or extracted from an alkaline aqueous solution with ethyl acetate and prepared as a solution containing about 2 mg/ml of drug. No significant differences between the R_F of the free base and those of the salts (especially the hydrochlorides) were observed. All drug solutions were freshly made and aliquots, 2–3 μl containing 4–6 μg of drug (except where otherwise stated in Table II), were applied approximately 1 cm apart to 20 × 10 cm silica gel 60 F_{254} HPTLC plates (Merck). For eluent IV the plates were dipped in 0.1 *M* potassium hydroxide methanolic solution and dried before application of the drugs. The quantity of drug applied was strictly dependent on the sensitivity towards the detection reagents. Amounts of 4–6 μg were usually sufficient to obtain spots which were clearly visible and had the same intensities as those of the reference compounds: cases where higher quantities of drugs were needed are explicitly indicated by footnotes in Table II.

The standardization procedure suggested by Stead *et al.*[3] for the correction of

TABLE I

STANDARDIZED TLC SYSTEMS

Silica gel 60 F_{254} HPTLC plates and saturated chambers were used throughout. Solutions of the four reference compounds were prepared to give a concentration of approximately 2 mg/ml of each compound.

No.	*Eluent mixture (v/v)*	*Reference compound*	$R_F \times 100$
I	Ethyl acetate–methanol–30% ammonia (85:10:5)	Morphine	25
		Strychnine	44
		Aminopyrine	70
		Cocaine	85
II	Cyclohexane–toluene–diethylamine (65:25:10)	Clobazam	15
		Aminopyrine	29
		Mebeverine	47
		Amitriptyline	60
III	Ethyl acetate–chloroform (50:50)	Caffeine	9
		Ketamine	24
		Flunitrazepam	44
		Prazepam	61
IV	Acetone*	Imipramine	20
		Pericyazine	37
		Aminopyrine	62
		Lidocaine	78

* Plates were dipped in 0.1 *M* potassium hydroxide methanolic solution and dried.

$R_F \times 100$ values was adopted throughout. A solution (2 μl) containing an appropriate mixture of reference compounds (see Table I) was applied at three separate positions along the baseline of each plate, together with the solution of the drugs. The solvents (100 ml) were placed into TLC tanks, which were sealed and allowed to equilibrate for at least 30 min before use. The systems were allowed to migrate 5 cm from the baseline. The use of shorter distances has been shown not to produce significant changes either in the corrected R_F values or in the reproducibility[15]. The solvent front was marked and the plates were air-dried. The drug detection was achieved by spraying first with 10% sulphuric acid, then with the Dragendorff spray reagent[3,13] and finally with acidified iodoplatinate solution[3,13].

The R_F values were measured independently in two laboratories where the eluent mixtures were freshly prepared using commercial solvents often provided by different companies; values were corrected according to the standardization procedure of Stead *et al.*[3]. The experimentally determined $R_F \times 100$ values were converted into the corrected values (R_Fc $\times$ 100) by a graphical method, using a six-point correction graph including the $R_F \times 100$ values of the four reference compounds, together with the 0,0 and 100,100 points. The R_Fc $\times$ 100 data for compounds 1–362 in eluent mixtures I–IV reported in Table II are averages of four determinations (two in each laboratory).

Principal components analysis

The PCA using the soft independent modelling of class analogy (SIMCA) method[16–19] and its applications for the identification of drugs by TLC in different

TABLE II

CORRECTED $R_FC \times 100$ VALUES IN ELUENTS I–IV, PRINCIPAL COMPONENTS SCORES, θ_1, θ_2, AND RESIDUAL STANDARD DEVIATIONS s_k AFTER 2 PC, FOR COMPOUNDS 1–362

No.	*Compound**	$R_FC \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
1	Acebutolol	41	0	0	6	−1.985	0.263	0.269
2	Acepromazine	77	33	1	32	0.101	−0.826	0.284
3	Acetylcodeine**	65	28	5	22	−0.393	−0.496	0.283
4	Adiphenine	88	61	5	66	1.688	−1.228	0.371
5	Ajmalicine	87	42	39	80	2.495	0.769	0.334
6	Ajmaline	60	9	1	15	−1.122	−0.105	0.444
7	Albuterol	26	0	0	6	−2.372	0.461	0.244
8	Alprenolol***	62	15	0	13	−1.025	−0.369	0.421
9	Amantadine	41	24	0	3	−1.596	−0.480	0.465
10	Ambroxol§	72	13	5	70	0.458	0.445	0.765
11	Amiloride§	28	0	0	3	−2.380	0.398	0.253
12	Amineptine	5	0	3	4	−2.873	0.825	0.738
13	7-Aminodesmethylflunitrazepam	59	1	10	65	−0.066	1.092	0.601
14	7-Aminoflunitrazepam	67	4	15	68	0.389	1.120	0.528
15	Aminophylline§	3	0	0	0	−3.084	0.691	0.738
16	Aminopyrine	70	29	12	62	0.734	0.160	0.272
17	Amiodarone	90	68	7	73	2.062	−1.300	0.475
18	Amitriptyline	82	60	2	25	0.623	−1.736	0.345
19	Amphetamine§	52	25	0	26	−0.839	−0.371	0.130
20	Antipyrine	52	7	11	45	−0.504	0.798	0.148
21	Atenolol	30	0	0	2	−2.348	0.359	0.256
22	Atropine	36	9	0	1	−2.045	0.003	0.341
23	Azanidazole	71	3	4	60	0.021	0.592	0.802
24	Bamethan§	44	6	0	−†	−1.579	0.206	0.108
25	Beclamide***,§	79	12	50	78	1.983	2.138	0.470
26	Benfluorex	86	44	19	75	1.872	−0.075	0.197
27	Benorylate§	69	0	31	77	0.973	1.911	0.303
28	Benoxinate	86	31	2	55	0.778	−0.565	0.553
29	Benzoxiquine§	83	27	31	75	1.798	0.907	0.111
30	Benzphetamine	90	75	35	83	3.141	−0.352	0.771
31	Benzydamine	76	46	0	14	−0.065	−1.454	0.411
32	Betahistine	22	8	0	0	−2.445	0.205	0.503
33	Biperiden***	91	74	10	76	2.340	−1.343	0.530
34	Bornaprine**	84	68	1	31	0.916	−1.961	0.316
35	Bromazepam	64	5	9	72	0.249	0.958	0.723
36	Bromhexine	92	77	80	87	4.513	1.269	1.857
37	Bromocriptine	79	6	17	77	0.968	1.087	0.702
38	Bromopride	61	2	0	19	−1.175	0.100	0.556
39	Brompheniramine	66	42	0	7	−0.536	−1.291	0.477
40	Brucine	35	4	0	2	−2.144	0.175	0.283
41	Buflomedil	60	21	0	11	−1.004	−0.544	0.348
42	Bupivacaine	86	49	18	80	2.037	−0.197	0.332
43	Butalamine	90	39	12	83	1.853	−0.139	0.570
44	Butamirate citrate	78	53	0	21	0.256	−1.600	0.306
45	Butethamate	88	64	2	63	1.604	−1.464	0.392
46	Butriptyline	89	70	3	57	1.650	−1.691	0.311
47	Caffeine	59	7	9	51	−0.258	0.706	0.347
48	Camazepam	81	17	39	79	1.853	1.572	0.217

TABLE II *(continued)*

No.	*Compound**	$R_Fc \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
49	Carbamazepine	61	4	21	66	0.355	1.395	0.271
50	Chlophedianol	81	48	3	49	0.875	−1.037	0.150
51	Chlordiazepoxide	53	4	7	56	−0.424	0.862	0.486
52	Chlormezanone§	75	5	29	73	1.088	1.562	0.356
53	Chloroquine	56	17	0	0	−1.400	−0.510	0.512
54	Chlorpheniramine maleate	63	41	0	7	−0.632	−1.222	0.468
55	Chlorpromazine	80	51	2	29	0.482	−1.395	0.210
56	Chlorprothixene	84	56	5	40	0.977	−1.349	0.136
57	Chromonar	74	32	0	21	−0.239	−0.929	0.346
58	Cimetidine	34	0	0	10	−2.087	0.405	0.109
59	Cinepazet maleate	66	7	9	68	0.259	0.824	0.650
60	Cinepazide maleate	40	0	0	13	−1.872	0.363	0.136
61	Cinnarizine	91	61	41	83	3.066	0.268	0.614
62	Clemastine	79	56	0	13	0.179	−1.800	0.475
63	Clobazam	78	15	46	78	1.906	1.915	0.360
64	Clobutinol	87	60	0	48	1.153	−1.592	0.149
65	Clofezone	72	26	0	23	−0.363	−0.702	0.365
66	Clomipramine	83	60	0	28	0.654	−1.786	0.232
67	Clonazepam	67	0	34	77	1.001	2.048	0.202
68	Clonidine	76	11	1	66	0.338	0.254	0.890
69	Clopenthixol	48	11	0	15	−1.421	−0.042	0.146
70	Cloperastine	89	63	2	60	1.552	−1.485	0.317
71	Clorazepate	73	7	36	75	1.301	1.812	0.203
72	Clothiapine	78	44	7	45	0.750	−0.781	0.014
73	Cocaine	85	49	4	68	1.399	−0.847	0.462
74	Codeine	42	11	0	7	−1.734	−0.062	0.245
75	Colchicine§	36	0	3	21	−1.737	0.625	0.043
76	Cropropamide§	78	37	41	74	2.104	1.035	0.492
77	Crotethamide§	75	32	34	73	1.726	0.951	0.276
78	Cyclizine	77	51	3	29	0.431	−1.319	0.235
79	Cyclopentamine	41	37	1	4	−1.307	−0.813	0.695
80	Cyclopentolate	73	39	3	43	0.381	−0.740	0.108
81	Cycrimine	89	74	9	73	2.202	−1.390	0.540
82	Cyproeptadine	79	51	1	22	0.291	−1.505	0.311
83	Delorazepam	75	8	37	75	1.398	1.793	0.239
84	Deptropine citrate	46	31	0	0	−1.396	−0.789	0.555
85	N-1-Desalkylflurazepam	75	6	35	76	1.327	1.790	0.263
86	Desipramine	48	24	0	4	−1.396	−0.560	0.398
87	Desmethyldiazepam***	73	5	35	75	1.237	1.834	0.239
88	Desmethylflunitrazepam	69	2	36	77	1.144	2.036	0.180
89	Dexetimide	76	29	6	61	0.709	−0.153	0.431
90	Diacetylmorphine	59	21	1	19	−0.845	−0.395	0.206
91	Diazepam	84	31	52	77	2.500	1.574	0.656
92	Dibenzepin	65	28	0	20	−0.566	−0.705	0.222
93	Dicyclomine	90	72	10	72	2.198	−1.320	0.469
94	Dihydrocodeine	39	8	0	4	−1.927	0.029	0.277
95	Dihydroergotamine§	49	0	0	42	−1.066	0.602	0.486
96	Dihydroergotoxine§	63	0	0	63	−0.289	0.676	0.905
97	Dimefline	70	25	0	42	−0.058	−0.411	0.340

(Continued on p. 156)

TABLE II *(continued)*

No.	*Compound**	$R_F c \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
98	Dimenhydrinate	78	51	0	27	0.337	−1.467	0.183
99	Dimethindene	63	41	0	8	−0.612	−1.209	0.450
100	Dimethophrine§	14	4	0	20	−2.330	0.675	0.630
101	Diphenhydramine	76	51	0	27	0.286	−1.441	0.184
102	Diphenoxylate	94	59	68	85	3.868	1.306	1.266
103	N-(1,2-Diphenylethyl)nicotinamide	77	6	24	70	0.965	1.285	0.459
104	α,α-Diphenyl-2-piperidinepropanol	91	68	40	83	3.170	0.025	0.718
105	Dipyridamole	47	2	2	63	−0.611	0.902	0.780
106	Dipyrone§	2	0	0	3	−3.051	0.741	0.762
107	Disopyramide	70	13	0	27	−0.579	−0.244	0.526
108	Disulfiram	86	52	83	83	3.893	2.145	1.703
109	Ditazol	54	4	9	65	−0.166	1.033	0.589
110	Dixyrazine	55	14	0	23	−1.026	−0.124	0.165
111	Domiodol**	16	0	0	4	−2.670	0.569	0.448
112	Domperidone	51	1	1	55	−0.712	0.743	0.668
113	Doxapram	78	32	7	71	1.041	−0.107	0.547
114	Doxepin	77	55	0	24	0.327	−1.609	0.271
115	Doxylamine	65	45	0	8	−0.486	−1.354	0.482
116	Droperidol	65	4	1	60	−0.196	0.532	0.798
117	Dropropizine**	39	3	1	33	−1.419	0.571	0.274
118	Ephedrine§§	38	10	0	5	−1.896	−0.004	0.277
119	Eprazinone	90	56	9	78	1.991	−0.812	0.473
120	Eprozinol	75	28	0	57	0.424	−0.381	0.550
121	Estazolam	57	2	5	45	−0.629	0.658	0.438
122	Etafenone	87	54	4	63	1.445	−1.083	0.339
123	Etamiphyllin	67	19	0	33	−0.425	−0.306	0.335
124	Ethambutol§	21	0	0	0	−2.620	0.453	0.377
125	Ethoheptazine	63	46	0	9	−0.499	−1.344	0.484
126	Ethosuximide	11	1	0	1	−2.839	0.568	0.581
127	Ethylmorphine	44	13	0	8	−1.625	−0.134	0.243
128	Etoperidone	69	18	1	45	−0.128	−0.118	0.447
129	Fazadinium bromide	80	27	41	71	1.909	1.265	0.411
130	Febutol**	80	12	22	75	1.200	1.056	0.471
131	Fenalamide	83	50	1	59	1.108	−1.072	0.380
132	Fendiline	90	65	11	76	2.173	−1.028	0.437
133	Fenfluramine	70	49	0	19	−0.065	−1.401	0.320
134	Fenspiride	44	0	0	28	−1.472	0.495	0.244
135	Fentanyl citrate	87	54	14	75	1.951	−0.566	0.333
136	Fentiazac	10	0	11	0	−2.609	1.004	0.826
137	Fipexide	84	23	19	74	1.408	0.557	0.407
138	Flavoxate	85	44	4	66	1.266	−0.725	0.456
139	Floctafenine	51	1	17	71	0.033	1.530	0.499
140	Flunarizine	91	54	35	81	2.735	0.228	0.361
141	Flunitrazepam	83	18	44	77	2.018	1.675	0.377
142	Fluphenazine	54	10	1	20	−1.159	0.007	0.231
143	Flurazepam	80	36	1	61	0.809	−0.596	0.521
144	Fominoben	84	25	48	80	2.341	1.640	0.442
145	Fonazine	73	20	1	51	0.131	−0.156	0.534
146	Furosemide§	7	0	2	2	−2.888	0.737	0.680
147	Glafenine	48	2	6	62	−0.498	1.024	0.644
148	Glaziovine**,§	55	8	1	35	−0.874	0.237	0.311

TABLE II *(continued)*

No.	*Compound**	$R_{FC} \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
149	Glipizide§	10	0	0	2	−2.864	0.623	0.581
150	Guaiapate	63	39	0	16	−0.491	−1.052	0.294
151	Guancthidine§§	1	0	0	0	−3.136	0.718	0.783
152	Haloperidol	81	19	0	59	0.451	−0.170	0.772
153	Heptaminol§§§	23	5	0	16	−2.158	0.477	0.401
154	Hexobendine	67	18	0	10	−0.899	−0.561	0.534
155	Hordenine	51	10	0	9	−1.481	−0.126	0.313
156	Hydromorphone	26	5	0	3	−2.338	0.277	0.358
157	Hydroquinidine	52	6	0	7	−1.570	−0.047	0.408
158	N-1-Hydroxyethylflurazepam	65	6	15	70	0.415	1.112	0.510
159	Hydroxyzine	62	13	0	48	−0.369	0.122	0.508
160	Imipramine	76	56	0	20	0.241	−1.674	0.351
161	Imolamine	77	23	0	43	0.105	−0.433	0.501
162	Indapamide§	71	1	53	81	1.711	2.715	0.414
163	Indoprofen§§	4	0	13	2	−2.671	1.181	0.962
164	Iodochlorhydroxyquin	29	0	12	20†	−1.696	1.036	0.371
165	Isoniazid§	37	0	2	23	−1.698	0.599	0.073
166	Isothipendyl	76	49	0	25	0.209	−1.407	0.204
167	Isoxsuprine	67	5	1	71	0.093	0.612	0.961
168	Josamycin	79	24	6	82	1.108	0.214	0.841
169	Ketamine	83	41	24	76	1.892	0.250	0.149
170	Ketocaine**	90	66	10	83	2.303	−1.008	0.585
171	Ketoconazole	54	0	0	16	−1.452	0.215	0.443
172	Ketotifen fumarate	67	31	1	15	−0.531	−0.845	0.343
173	Labetalol	32	0	0	22	−1.901	0.580	0.172
174	Lefetamine	85	61	6	56	1.439	−1.275	0.241
175	Levallorphan tartrate	81	28	0	52	0.480	−0.522	0.550
176	Levorphanol tartrate	52	22	0	4	−1.330	−0.554	0.389
177	Lidocaine	84	41	23	78	1.931	0.224	0.180
178	Lisuride§	67	6	1	62	−0.067	0.471	0.816
179	Loperamide	81	15	0	49	0.178	−0.176	0.760
180	Lorajmine§	69	15	0	50	−0.112	−0.005	0.578
181	Lorazepam	47	2	28	72	0.263	1.971	0.335
182	Lormetazepam	61	11	41	71	1.121	1.987	0.332
183	Lysergide	66	2	2	50	−0.378	0.491	0.698
184	Maprotiline	44	27	0	3	−1.463	−0.608	0.477
185	Mazindol	58	12	0	22	−1.006	−0.117	0.280
186	Mebendazole§§§	72	2	17	69	0.554	1.198	0.588
187	Mebeverine	89	47	3	63	1.339	−0.940	0.418
188	Mebhydroline§	77	37	1	33	0.196	−0.931	0.204
189	Meclofenoxate§§§	71	33	0	34	−0.041	−0.759	0.150
190	Medazepam	85	49	44	77	2.648	0.737	0.639
191	Melitracen	84	58	2	28	0.696	−1.667	0.276
192	Meperidine	72	42	0	19	−0.144	−1.222	0.277
193	Mephentermine§	51	39	0	5	−1.019	−1.029	0.552
194	Mepivacaine	76	38	6	65	0.956	−0.368	0.433
195	Mepixanthone	81	36	0	55	0.689	−0.720	0.464
196	Metergoline§	75	9	0	41	−0.247	−0.019	0.719
197	Methadone	87	66	0	51	1.325	−1.731	0.246

(Continued on p. 158)

TABLE II *(continued)*

No.	*Compound**	$R_Fc \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
198	Methadone metabolite	90	74	5	48	1.626	−1.859	0.300
199	Methamphetamine	50	35	0	5	−1.119	−0.898	0.504
200	Methaqualone	84	40	57	77	2.802	1.493	0.899
201	Methixene	78	54	1	20	0.282	−1.605	0.360
202	Methotrimeprazine	86	57	0	51	1.131	−1.453	0.216
203	Methoxyphenamine	51	32	0	6	−1.130	−0.811	0.440
204	Methylergonovine§§	44	0	0	22	−1.591	0.421	0.190
205	Methylphenidate	77	44	1	44	0.544	−1.001	0.147
206	Methysergide	49	2	1	36	−1.121	0.506	0.344
207	Metoclopramide	59	3	0	17	−1.247	0.073	0.503
208	Metoprolol§	54	13	0	10	−1.328	−0.242	0.324
209	Metronidazole§	51	0	0	53	−0.797	0.711	0.673
210	Mexiletine	68	30	0	45	0.044	−0.495	0.300
211	Mianserin	80	48	7	45	0.877	−0.925	0.053
212	Miconazole	84	19	14	60	0.923	0.318	0.510
213	6-Monoacetylmorphine***	55	10	0	14	−1.279	−0.118	0.321
214	Moprolol	51	14	0	6	−1.466	−0.281	0.342
215	Morclofone	82	32	10	66	1.126	−0.111	0.395
216	Morphazinamide	55	8	3	39	−0.741	0.360	0.302
217	Morphine	25	1	0	4	−2.419	0.420	0.295
218	Moxaverine§	85	46	60	82	3.120	1.476	0.982
219	Moxisylyte	79	40	2	31	0.290	−1.034	0.226
220	Muzolimine	55	0	9	63	−0.255	1.113	0.589
221	Nadolol§	29	1	0	1	−2.375	0.330	0.288
222	Nafronyl	86	56	1	54	1.198	−1.350	0.249
223	Nalorphine	33	3	1	47	−1.297	0.824	0.615
224	Naloxone§	42	13	14	75	0.024	1.235	0.795
225	Naphazoline	42	4	0	1	−1.984	0.070	0.354
226	Nefopam	69	40	1	19	−0.232	−1.087	0.279
227	Nicametate	79	46	1	38	0.514	−1.161	0.059
228	Nicergoline	80	29	4	47	0.481	−0.452	0.381
229	Niceritrol§§	67	1	0	38	−0.662	0.285	0.699
230	Nicotine	68	42	1	23	−0.141	−1.083	0.219
231	Nifedipine§§	80	1	55	78	1.937	2.632	0.626
232	Nikethamide	67	23	13	55	0.433	0.326	0.150
233	Nimorazole	64	5	1	48	−0.440	0.367	0.623
234	Nitrazepam	57	0	33	69	0.558	2.045	0.014
235	Nitrofurazone§§	48	1	4	56	−0.689	0.906	0.596
236	Nomifensine	74	17	2	54	0.187	−0.007	0.602
237	Norcyclizine	77	49	2	29	0.367	−1.297	0.190
238	Nordazepam	73	5	37	75	1.290	1.907	0.238
239	Nortriptyline	57	36	1	6	−0.873	−0.971	0.482
240	Noscapine	85	30	37	78	2.126	1.050	0.196
241	Orphenadrine	79	53	2	29	0.494	−1.441	0.219
242	Otilonium bromide§	2	0	0	0	−3.110	0.704	0.760
243	Oxazepam	46	3	23	60	−0.115	1.622	0.241
244	Oxeladin citrate	83	54	0	23	0.443	−1.671	0.301
245	Oxethazaine	58	12	1	46†	−0.504	0.216	0.433
246	Oxolamine citrate	87	61	6	67	1.709	−1.166	0.384
247	Oxprenolol	57	17	0	10	−1.176	−0.399	0.344

TABLE II *(continued)*

No.	*Compound**	$R_FC \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
248	Oxycodone	76	33	0	44	0.286	−0.701	0.313
249	Oxyfedrine	79	21	2	60	0.510	−0.117	0.671
250	Oxymetazoline	32	2	0	2	−2.259	0.274	0.269
251	Oxymorphone	38	15	1	41	−1.063	0.330	0.506
252	Pancuronium bromide§	1	1	0	0	−3.117	0.688	0.802
253	Papaverine	74	14	24	68	0.997	1.064	0.236
254	Pentazocine	80	23	2	46	0.296	−0.362	0.532
255	Pentifylline	66	11	20	66	0.588	1.086	0.249
256	Pentoxifylline	54	4	6	57	−0.405	0.824	0.535
257	Perhexiline maleate	73	63	1	12	0.162	−1.903	0.608
258	Pericyazine	64	7	1	37	−0.621	0.173	0.500
259	Perphenazine	54	9	0	17	−1.264	−0.038	0.277
260	Phenacetin§	76	2	35	76	1.278	1.895	0.361
261	Phenazocine	78	25	7	68	0.851	0.062	0.570
262	Phendimetrazine	76	43	6	36	0.475	−0.873	0.139
263	Phendimetrazine bitartrate	73	43	5	34	0.331	−0.895	0.145
264	Phenformin***§	3	0	0	0	−3.084	0.691	0.738
265	Phenindamine	81	52	4	40	0.798	−1.229	0.079
266	Pheniramine maleate	61	41	0	6	−0.704	−1.208	0.489
267	Phenmetrazine	56	19	0	13	−1.105	−0.408	0.259
268	Phenpyramine**	72	4	0	18	−0.873	−0.116	0.793
269	Phentolamine	44	1	0	3	−1.948	0.157	0.367
270	Phenyltoloxamine	77	43	4	38	0.487	−0.936	0.063
271	Pilocarpine	50	0	0	12†	−1.634	0.218	0.382
272	Piminodine citrate	89	45	6	78	1.679	−0.585	0.606
273	Pimozide	77	7	5	75	0.574	0.617	0.963
274	Pinaverium bromide	7	0	0	2	−2.941	0.663	0.648
275	Pinazepam	87	34	66	81	3.088	2.011	1.022
276	Pindolol	54	4	1	10	−1.469	0.060	0.443
277	Pipamperone	52	4	0	9	−1.567	0.037	0.404
278	Pipazethate	60	22	0	11	−0.985	−0.574	0.341
279	Piperidolate	86	60	7	72	1.790	−1.024	0.466
280	Pirenzepine	21	0	0	1	−2.600	0.466	0.368
281	Piretanide§	2	1	4	1	−2.964	0.835	0.844
282	Piroxicam	15	0	33	67	−0.565	2.575	1.012
283	Pizotyline maleate	74	51	3	12	0.018	−1.489	0.540
284	Prajmaline	74	28	1	24	−0.228	−0.738	0.372
285	Prazepam	86	41	61	81	3.059	1.634	0.954
286	Prazosin	63	2	3	68	−0.073	0.789	0.866
287	Prenylamine lactate	87	59	6	65	1.632	−1.131	0.336
288	Pridinol methanesulphonate	88	65	5	74	1.921	−1.247	0.541
289	Prifinium bromide	2	0	0	3	−3.051	0.741	0.762
290	Procaine	78	9	1	48	−0.004	0.065	0.789
291	Prochlorperazine dimaleate	64	40	1	9	−0.559	−1.144	0.453
292	Procyclidine	84	68	2	38	1.081	−1.837	0.283
293	Proglumetacin dimaleate	78	10	1	62	0.291	0.208	0.884
294	Prolintane	83	70	1	35	1.006	−1.957	0.334
295	Promazine	72	48	1	17	−0.045	−1.386	0.369
296	Promethazine	74	44	2	28	0.177	−1.122	0.174

(Continued on p. 160)

TABLE II *(continued)*

No.	*Compound**	$R_Fc \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
297	Propanidid	74	27	44	73	1.875	1.480	0.489
298	Propoxyphene	87	66	5	66	1.756	−1.362	0.424
299	Propranolol	55	13	1	10	−1.275	−0.218	0.359
300	Propylhexedrine§	42	43	1	4	−1.169	−1.003	0.785
301	Propyphenazone	80	38	60	79	2.782	1.740	0.961
302	Protriptyline	45	27	0	3	−1.437	−0.621	0.468
303	Proxazole citrate	88	64	8	71	1.923	−1.144	0.426
304	Pyridinol carbamate	64	2	8	70	0.127	0.985	0.757
305	Pyritinol	7	0	2	15†	−2.630	0.897	0.703
306	Quinidine	57	6	0	10	−1.381	−0.076	0.476
307	Quinine	81	29	6	66	0.937	−0.157	0.542
308	Racemethorphan	58	49	1	7	−0.585	−1.354	0.625
309	Ranitidine	32	0	0	6	−2.217	0.382	0.182
310	Reproterol§§	14	0	0	25†	−2.305	0.854	0.614
311	Reserpine	86	23	8	80	1.284	0.200	0.807
312	Ritodrine§	44	0	0	34†	−1.353	0.569	0.331
313	Rociverine	90	72	17	81	2.563	−0.951	0.545
314	Scopolamine	54	10	0	35	−0.889	0.155	0.295
315	Sotalol§§	35	0	0	8	−2.100	0.367	0.150
316	Sparteine	44	71	0	2	−0.661	−1.915	1.255
317	Stanozolol	66	8	12	64	0.279	0.855	0.492
318	Strychnine	44	12	2	5	−1.650	−0.068	0.351
319	Succiphylline**	63	6	4	58	−0.169	0.585	0.638
320	Sulfinpyrazone	18	0	0	40	−1.905	0.987	0.742
321	Suloctidil	86	41	3	57	1.031	−0.798	0.384
322	Sulpiride	44	0	0	14	−1.749	0.322	0.218
323	Sultopride	55	4	0	16	−1.351	0.084	0.396
324	Syrosingopine§	86	4	11	80	1.010	0.869	1.023
325	Temazepam	67	14	43	73	1.424	1.918	0.348
326	Tenitramine**	10	0	0	18†	−2.547	0.821	0.640
327	Terfenadine§	83	19	1	58	0.509	−0.172	0.766
328	Tetrahydrozoline	39	11	0	2	−1.911	−0.084	0.335
329	Tetramisole	76	25	4	60	0.561	−0.121	0.524
330	Thenalidine	76	47	0	19	0.053	−1.422	0.314
331	Thenyldiamine§	76	47	0	24	0.152	−1.360	0.217
332	Theobromine§§	36	0	2	12	−1.942	0.477	0.134
333	Theophylline§§	12	0	6	28	−2.137	1.139	0.689
334	Thiethylperazine	67	38	0	9	−0.545	−1.162	0.427
335	Thiopropamine**	85	53	5	54	1.224	−1.101	0.155
336	Thioridazine	79	51	0	23	0.284	−1.530	0.265
337	Tiapride**	52	2	0	18	−1.426	0.207	0.344
338	Ticlopidine	85	71	78	84	4.108	1.428	1.830
339	Timolol§	60	13	0	23	−0.916	−0.161	0.304
340	Tipepidine citrate	84	56	5	60	1.373	−1.102	0.292
341	Trazodone	75	18	4	60	0.404	0.098	0.615
342	Tretoquinol	36	0	0	10	−2.035	0.378	0.120
343	Triazolam	53	3	4	26	−1.117	0.410	0.278
344	Trifluoperazine	70	39	1	15	−0.304	−1.120	0.362
345	Trifluperidol	86	20	4	67	0.864	−0.020	0.805
346	Triflupromazine	85	52	2	41	0.867	−1.343	0.065
347	Trihexyphenidyl§	89	73	7	73	2.130	−1.434	0.546

TABLE II *(continued)*

No.	*Compound**	$R_Fc \times 100$				θ_1	θ_2	s_k
		I	*II*	*III*	*IV*			
348	Trimebutine maleate**	88	54	52	82	3.132	0.906	0.840
349	Trimethoprim§	48	0	0	16	−1.607	0.294	0.297
350	Trimipramine maleate	87	66	4	56	1.531	−1.522	0.280
351	Trithiozine	84	27	58	77	2.586	1.913	0.800
352	Tritoqualine	88	35	67	83	3.199	2.030	1.024
353	Valnoctamide§§	71	5	30	72	1.012	1.651	0.252
354	Verapamil	83	30	4	61	0.854	−0.348	0.538
355	Viloxazine	47	10	2	10	−1.511	0.012	0.273
356	Viminol	90	66	59	84	3.635	0.809	1.170
357	Vincamine	81	41	11	59	1.156	−0.413	0.147
358	Viquidil	35	6	0	2	−2.107	0.116	0.299
359	Xylometazoline	41	12	0	4	−1.801	−0.115	0.306
360	Yohimbine	72	8	4	69	0.318	0.543	0.846
361	Zipeprol	72	32	4	60	0.588	−0.274	0.438
362	Zolimidine	64	2	15	68	0.274	1.218	0.526

* Nomenclature according to the *Merck Index*[14] except where otherwise stated. Quantity of drug in the range 4–6 μg except where otherwise stated.

** Nomenclature according to Chemical Abstracts.

*** Nomenclature according to Clarke[13].

§ Quantity of drug in the range 7–15 μg.

§§ Quantity of drug in the range 16–30 μg.

§§§ Quantity of drug in the range 30–50 μg.

† The chromatographic spot showed an elongated shape.

eluent systems[10–12] have been presented in detail. In the present instance, the matrix Y with the elements y_{ik} contains R_Fc values, where index i is used for the eluent mixtures (variables) and index k for the compounds (objects). From this data matrix, the number of significant product terms, A, and then the parameters α_i, β_{ia} and θ_{ak} in eqn. 1 are estimated by minimizing the sum of the cross validated squared residuals, ε_{ik}:

$$y_{ik} = \alpha_i + \sum_{a=1}^{A} \beta_{ia}\,\theta_{ak} + \varepsilon_{ik} \qquad (1)$$

In this model, α_i and β_{ia} are constants which are only dependent on the eluent mixtures and θ_{ak} are the compound-dependent parameters. The deviations from the model are expressed by the residuals ε_{ik}, which include also the experimental errors in the determination of the R_Fc values.

Before the PCA computation, the eluent parameters were autoscaled (see, *e.g.*, ref. 19), *i.e.*, the variables were given the same variance (unity). With this scaling, all variables were given the same initial importance in the PCA, so that the model chooses the relative importance of each eluent system when defining the components according to their information content. We are aware that other authors prefer to weight variables according to the measurement precision. However, we have given reasons for our choice: "the use of multivariate methods such as PCA, where all objects are

to be described at the same time by all variables, renders less dramatic the problem of reproducibility, since the experimental error gets lost in the residuals together with the error due to approximation of the mathematical model"[20].

RESULTS AND DISCUSSION

The use of HPTLC plates for the R_F determination has many advantages such as a higher reproducibility, a shorter analysis time (5 min as compared to 20–30 min) due to an acceptable separation over a developing distance of only 5 cm, which will result also in an improved sensitivity (3–4 μg as compared to 5–10 μg) due to the reduced diffusion of the spot. The reproducibility using the corrected R_F values according to the standardization procedure of Stead *et al.*.[3] (*cf.*, Experimental) is always $\leqslant 7\%$ for non-biological samples.

The examination of extracts from biological fluids and tissues or from post-mortem samples in various stages of decomposition is complicated by the interference from the biological matrix which alters the values of the chromatographic data[21]. In this case we suggest a preliminary chromatographic purification (and separation) using eluent I.

Thus, after elution, it is possible to scratch the spots from the plate, to separate from the silica gel by extraction with methanol, to concentrate the solution and to perform the TLC analysis for each substance in all four eluent systems. In order to carry out the PCA, the R_Fc values were arranged into a matrix (see Table II) with the compounds as "objects" and the eluent mixtures as "variables". Each of the 1448 elements of the matrix is indicated in eqn. 1 as y_{ik}.

The variables (R_Fc values for each eluent mixture) were first autoscaled[19]. Each element was multiplied by the weighting typical of the eluent (the reciprocal of the variable standard deviation) in order to give unit variance to each eluent mixture. The weightings for the individual variables I–IV are recorded in Table III.

The PCA of the data matrix gave a model comprising two significant principal components. A third component, still significant according to the cross validation technique[18], was not taken into account because of the small number of original variables. The first component explains 47% of the total variance and the second one a further 26%; the planar model thus accounts for 73% of the total variance. The values of α, β_1 and β_2 are recorded in Table III, while θ_1 and θ_2 values (the "scores" for compounds 1–362) are listed in Table II, together with the residual standard deviations, s_k, after two principal components.

In this paper we do not use the refinement procedure based on reweighting

TABLE III

WEIGHTINGS, α, β_1 AND β_2 FOR VARIABLES (ELUENT MIXTURES) I–IV

Variable (eluent mixture)	*Weighting*	α	β_1	β_2
I	0.0448	2.843	0.576	−0.295
II	0.0444	1.110	0.420	−0.662
III	0.0617	0.561	0.434	0.597
IV	0.0359	1.478	0.551	0.344

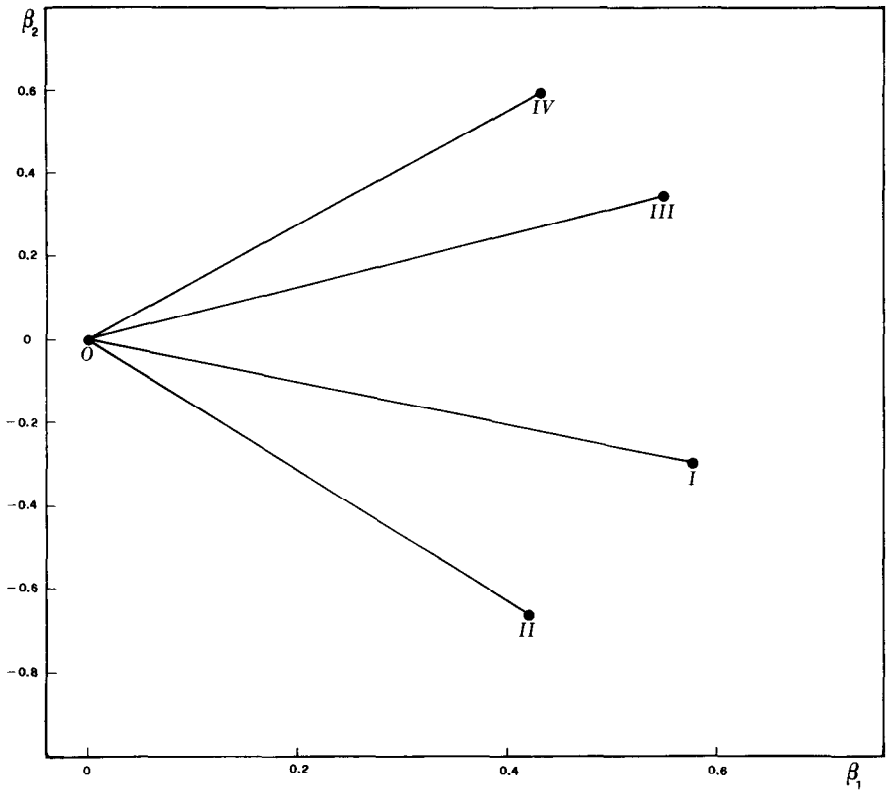


Fig. 1. Plot of β_2 *vs.* β_1 for variables (eluents) I–IV; 0 indicates origin (0,0).

with modelling powers as adopted in our previous work. We are aware that any reweighting procedure is somewhat arbitrary, and in view of the slight improvement in the identification ability of the model we now suggest that the analysis be limited to simple PCA[20].

Fig. 1, a plot of β_2 *vs.* β_1, shows that eluents I–IV, which lie along different directions with respect to the origin (0,0), have, in the present instance, different information contents, paralleling the trend already observed for 55 drugs[12].

Fig. 2, a plot of θ_2 *vs.* θ_1 for the 362 compounds examined, is the basis for the identification of unknowns (see below). However, a careful inspection of this figure provides also interesting insights into the "zones" where substances characterized either by analogous chemical structures or by similar pharmacological activities are grouped. Benzodiazepines are characterized by θ_1 values in the range -0.5 to 3.2 and θ_2 values in the range 0.7–2.1. Their location in the plot depends on the nature of the substituent, especially when attached at the 1-position.

A peculiar behaviour is shown by flurazepam (143), which has a much lower θ_2 value, probably due to the presence of a terminal diethylamino group at nitrogen-1. Low θ_2 values are exhibited by a great number of substances (almost all antihistamines and phenothiazinic tranquillisers and many antidepressants) containing similar groups as "predominant" substituents. Phenothiazinic tranquillisers where

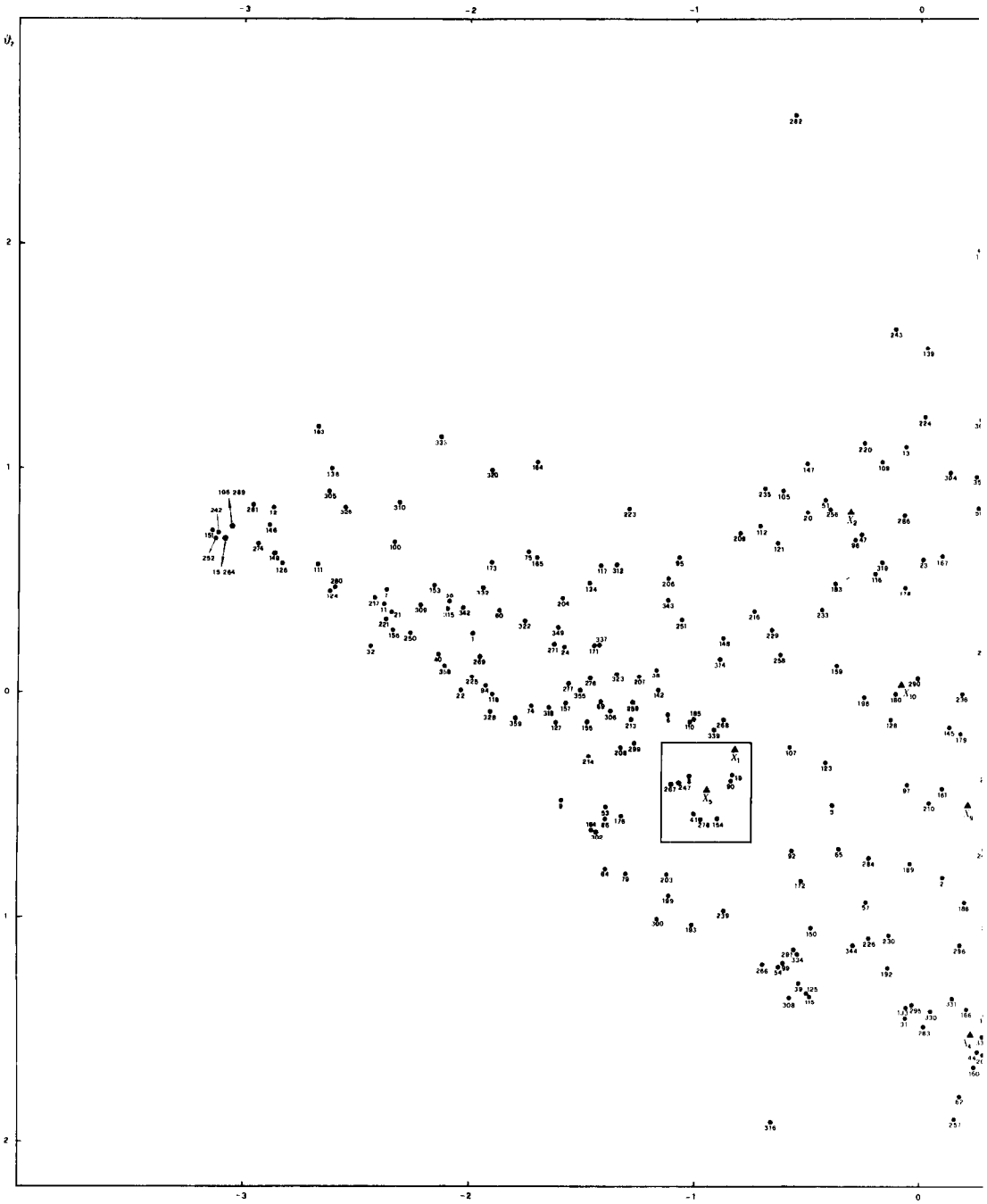
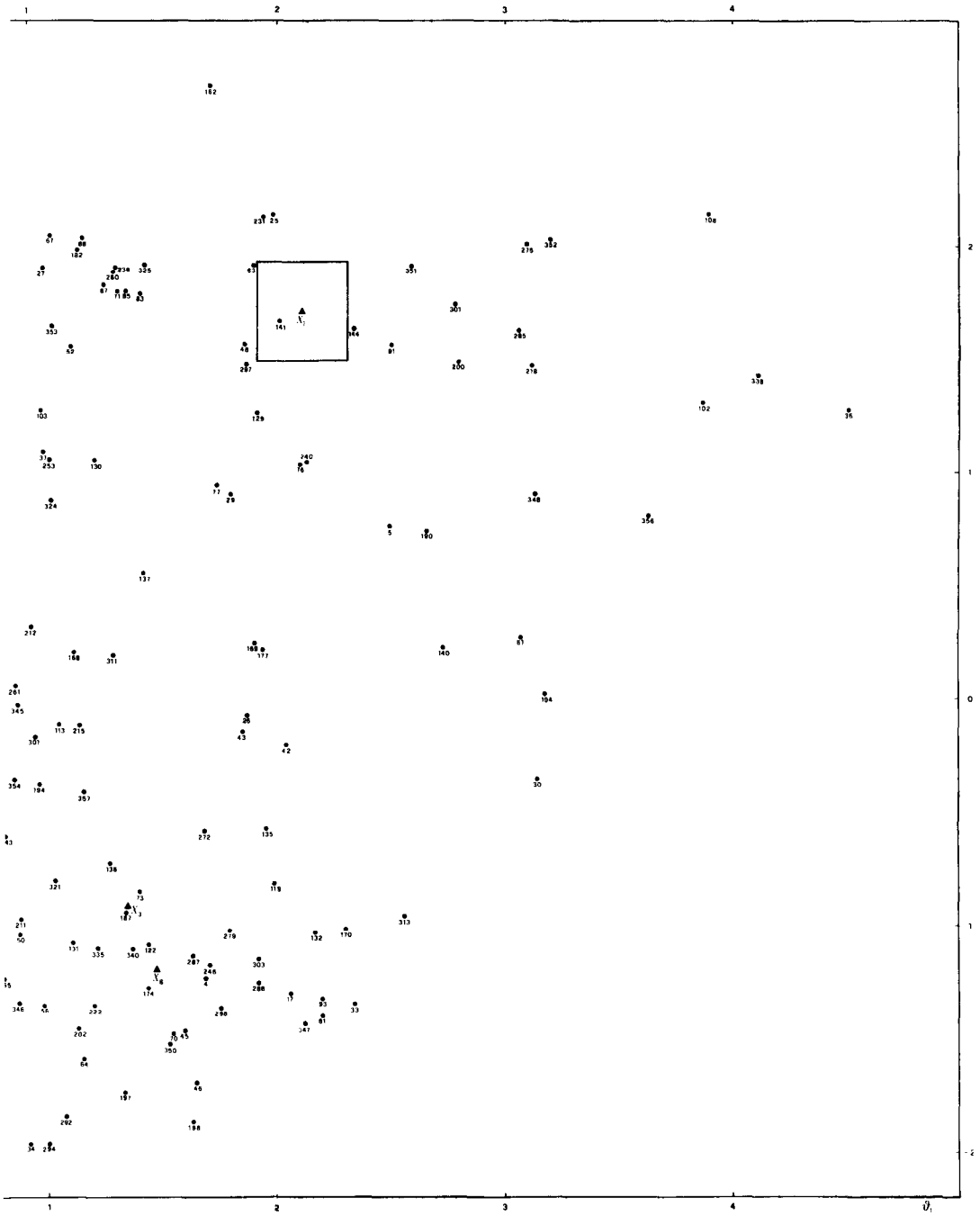


Fig. 2. Plot of θ_2 *vs.* θ_1 for compounds 1–362 (●) and of t_1 and t_2 for pseudo-unknowns X_1–X_{10} (▲). For X_5 and



confidence rectangles" are also reported.

the terminal diethylamino group is replaced by an hydroxyl (69, 110, 142, 258 and 259) exhibit much lower θ_1 values, being shifted towards the zone typical of substances with hydroxyl groups.

Compounds having the same base skeleton as morphine (217, 156, 94, 74 and 127) and synthetic derivatives with similar structures such as 176 and 308 lie along the same line which represents the lower left limit of the populated zone of Fig. 2. The position along this line is determined by the presence of one or more hydroxyl groups, which causes a shift toward the left, *i.e.*, lower θ_1 values. The lack of an hydroxyl group or partial or total transformation into the corresponding acetyl derivatives (213, 90, 3) causes a shift towards higher θ_1 values.

The replacement of a nitrogen methyl group in compounds 217, 156 and 176 with an allyl characteristic of antagonists (in 223, 224 and 175 respectively) shifts the latter derivatives towards higher values of both θ_1 and θ_2.

Identification of unknowns

The identification of unknowns, provided the unknown is one of the 362 compounds in the data set, can be attempted by measuring the corrected R_Fc values in the four eluents and fitting them with the PC model.

The t_1 and t_2 values for each unknown are given by eqns. 2 and 3 respectively

$$t_1 = 0.576\,(0.0448 \times 100\,R_F c_{\mathrm{I}} - 2.843) + 0.420\,(0.0444 \times 100\,R_F c_{\mathrm{II}} - 1.110) + 0.434\,(0.0617 \times 100\,R_F c_{\mathrm{III}} - 0.561) + 0.551\,(0.0359 \times 100\,R_F c_{\mathrm{IV}} - 1.478) \quad (2)$$

$$t_2 = -0.295\,(0.0448 \times 100\,R_F c_{\mathrm{I}} - 2.843) - 0.662\,(0.0444 \times 100\,R_F c_{\mathrm{II}} - 1.110) + 0.597\,(0.0617 \times 100\,R_F c_{\mathrm{III}} - 0.561) + 0.344\,(0.0359 \times 100\,R_F c_{\mathrm{IV}} - 1.478) \quad (3)$$

which can easily be simplified to:

$$t_1 = 0.0258\,(100\,R_F c_{\mathrm{I}} - 63.48) + 0.0186\,(100\,R_F c_{\mathrm{II}} - 25) + 0.0268\,(100\,R_F c_{\mathrm{III}} - 9.09) + 0.0198\,(100\,R_F c_{\mathrm{IV}} - 41.17) \quad (4)$$

$$t_2 = -0.0138\,(100\,R_F c_{\mathrm{I}} - 63.48) - 0.0294\,(100\,R_F c_{\mathrm{II}} - 25) + 0.0368\,(100\,R_F c_{\mathrm{III}} - 9.09) + 0.0123\,(100\,R_F c_{\mathrm{IV}} - 41.17) \quad (5)$$

The values for the unknown substance can be fitted into the "scores" plot (Fig. 2) to select the candidates for its identification.

The selection of candidates is done by defining a region of statistical relevance around the t values obtained for the unknown. For this purpose we measured the R_F values for 43 pseudo-unknowns representative of "good" (141) and "bad" (101) ones as well as of compounds which can hardly be distinguished by TLC (19 and 90). For each of the pseudo-unknowns we then determined the differences between their experimental t_1 and t_2 values and their "true" values reported in Table II. The averages

TABLE IV

$R_F c \times 100$ VALUES IN ELUENTS I–IV, t_1 AND t_2 VALUES AND "CANDIDATES" FOR UNKNOWN SAMPLES X_1–X_{10}

Unknown	$R_F c \times 100$				t_1	t_2	*"Candidates" at 99% confidence level*	*Compound*
	I	*II*	*III*	*IV*				
X_1	54	21	0	28	−0.823	−0.254	19, 90, 339, 268, 185, 110	19
X_2	56	6	9	53	−0.314	0.799	256, 47, 51, 96, 20, 147, 109	47
X_3	85	51	5	62	1.342	−0.943	187, 73, 340, 122, 335, 138	73
X_4	75	54	2	20	0.228	−1.526	336, 82, 44, 101, 166, 98, 201, 114, 160, 331, 330, 78	82
X_5	59	20	0	16	−0.950	−0.438	8, 90, 19, 41, 247, 278, 154, 267	90
X_6	82	53	0	25	0.435	−1.601	244, 114, 98, 82, 241, 336, 201, 44, 55, 101, 160, 18	101
X_7	83	19	45	79	2.104	1.705	141	141
X_8	86	60	8	55	1.477	−1.197	122, 340, 287, 174, 187	174
X_9	79	25	0	44	0.213	−0.505	161, 254, 210, 248, 120	254
X_{10}	79	9	0	44	−0.084	−0.034	180, 128, 290, 196	290

of these differences are 0.075 in both cases and their standard deviations are 0.050 and 0.058 for t_1 and t_2 respectively. Consequently, by means of appropriate student t values, we can conclude that there is a 95% probability of finding the "true" compound within ± 0.16 t_1 and ± 0.17 t_2 from the position of the unknown on the "scores" plot (Fig. 2) and that this probability is increased to 99% when the interval is ± 0.20 t_1 and ± 0.22 t_2.

A few illustrative examples of identification of unknowns (a complete list of t_1 and t_2 values for all 43 pseudo-unknowns is available on request from the authors) are reported in Table IV, which also lists the possible candidates included in the 99% "confidence rectangle" defined as before. The unknowns are reported as triangles in Fig. 2, which also depicts the 99% "confidence rectangles" for X_5 (90) and X_7 (141). The number of candidates obviously depends upon the number of compounds with similar TLC properties included in the set.

CONCLUSIONS

This work confirms the validity of PCA as a suitable statistical approach for the treatment of TLC data in different eluent systems aimed at the identification of drugs. The application of PCA, which reduces the number of variables, allows a graphical representation of all compounds in a two-dimensional space, *i.e.*, Fig. 2, and represents a great advantage over previous approaches using graphical representations[1,2] or tables[3].

Standardized R_F data in four eluent systems appropriately selected to extract the maximum information available from TLC data[1,2] are not sufficient to achieve unambiguous identification.

However, in the present instance, the reduction of the range of inquiry to a few candidates is, in the authors' opinion, satisfactory when account is taken of the cost, the time and the analytical instrumentation required by TLC measurements and

of the simplicity of the calculations involved. This approach is of great practical importance when financial resources, time and sophisticated analytical instrumentation are not available. Measurements of a different nature, such as gas chromatographic (GC) data, are needed to attempt unambiguous identification of unknowns. Further work on the application of PCA to both TLC and GC data is in progress.

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