CHROM. 5704

Identification of substances by thin-layer chromatography in one solvent on a range of layers

For most purposes a single chromatogram does not give sufficient information to permit identification of the substances present in a sample. Chromatographic systems of identification have thus used a series of solvents of different properties together with a number of reagents and in some cases identification can be achieved in this way.

FRANC AND MICHAILOVA[1] proposed an interesting scheme for identification in gas chromatography. Four columns are used in parallel in a single gas chromatograph, and a single sample is applied and run simultaneously on all four columns using a suitable sample splitter. When columns with different stationary phases are used, a "chromatographic spectrum" can be obtained consisting of four retention times for each constituent, and evidently thus permits a better characterisation than one single column.

We felt that this idea could be also applied in flat bed chromatography, especially as a wide range of thin layers is now commercially available and thus similar results can be obtained in different laboratories. The present note describes the results obtained with some alkaloids and with some indicator dyes.

TABLE I

R_F VALUES OF ALKALOIDS ON VARIOUS THIN LAYERS

Solvent: *n*-Butanol–acetic acid–water (4:1:2).

Compound	*Cellulose (Carlo Erba)*	*Acetylated cellulose AC-10 (MN)*	*Aluminium oxide (Carlo Erba)*	*Silica gel (Eastman)*	R_F *"word"*
Tropine	0.38	0.57	0.53	0.17	FLKD
Atropine	0.68	0.83	0.60	0.35	NQLG
Homatropine	0.64	0.76	0.57	0.33	MPLG
Belladonine	0.20	0.98	0.42	0.05	DTIA
Cocaine	0.69	0.90	0.69	0.30	NSNF
Scopolamine·HCl	0.46	0.76	0.46	0.28	JPJF
Hyoscyamine	0.68	0.90	0.68	0.33	NTNG
Tropococaine	0.46	0.89	0.72	0.44	JROJ
Narceine	0.68	0.98	0.55	0.25	NTKE
Morphine	0.32	0.98	0.46	0.25	GTJE
Papaverine	0.83	0.94	0.83	0.63	QSQH
Cortarnine	0.43	0.66	0.52	0.22	INKE
Narcotine	0.84	0.97	0.84	0.60	QTQL
Heroine	0.60	0.89	0.60	0.35	LRLG
Apomorphine	0.56	0.90	0.47	0.44	LRFJ
Hydrastine	0.78	0.93	0.71	0.48	PSOF

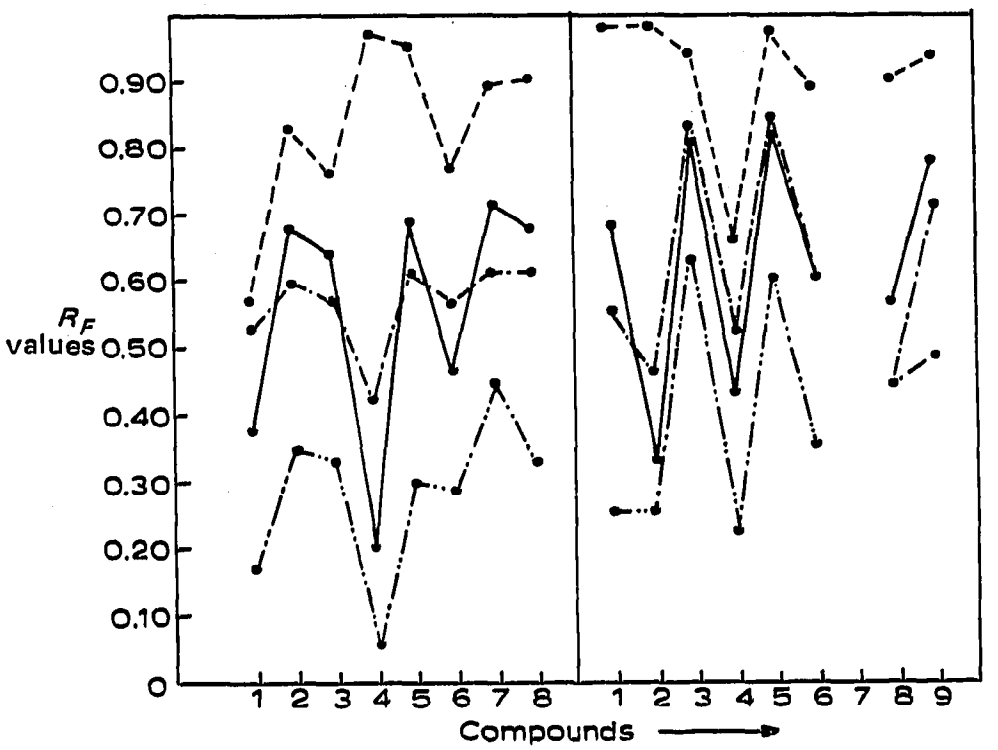

Fig. 1. R_F values of alkaloids on various layers. Each line joins R_F values on the same support. – – – –, Acetylated cellulose AC-10 MN;————, cellulose (Carlo Erba); –·–·–·–, aluminium oxide (Carlo Erba); ––––––––, silica gel (Eastman). The Tropan alkaloids (left hand graph) are numbered as follows: 1 = Tropine, 2 = atropine, 3 = homatropine, 4 = belladonine, 5 = cocaine, 6 = scopolamine·HCl, 7 = tropococaine, 8 = hyoscyamine. The morphine alkaloids (right hand graph) are numbered as follows: 1 = Narceine, 2 = morphine, 3 = papaverine, 4 = cotarnine·HCl, 5 = narcotine, 6 = heroine, 7 = apocodeine (yields many spots and thus not included in the graph), 8 = apomorphine.

Flat bed chromatography has several draw-backs in such a scheme compared to gas chromatography. R_F values have to be in the range of 0.05 to 0.9 for most compounds on all layers and preferably in the region of 0.3–0.7; also the change of support must not result in very high or very low R_F values, otherwise no advantage is gained. Furthermore, no better identification is obtained if all substances increase or decrease their R_F values by the same or similar degree from one layer to another. Thus results are best when different sequences are obtained in the measurable range for the whole group of substances studied.

Table I shows the R_F values of some of the common alkaloids with *n*-butanol–acetic acid–water, on cellulose, acetylated cellulose, alumina and silica gel. Fig. 1 shows graphically the R_F values of the various layers. This graph clearly shows that there are several inversions of the sequence.

Another way of examining the specificity of the procedure is to give a letter of the alphabet to each R_F interval of 0.05 (starting with A at R_F 0–0.05) as suggested by MACEK[2] and to examine whether the words so formed are identical for two compounds. In this case, as shown in the last column of Table I, there are no two identical words.

Table II and Fig. 2 show the R_F values obtained with a group of sulfonphthalein and other indicators using six different layers. Again amongst the nine compounds

TABLE II

R_F VALUES OF INDICATOR DYES

Solvent: Ethyl acetate–methanol–5 *N* ammonium hydroxide (80:10:10).

Compound	*Cellulose (Carlo Erba)*	*Cellulose MN*	*Acetylated cellulose AC-10 MN*	*DEAE cellulose MN*	*Aluminium oxide (Carlo Erba)*	*Silica gel (Eastman)*	R_F *"word"*
Bromocresol purple	0.24	0.15	0.45	0.04	0.00	0.03	ECIAA
Ortho-cresol red	0.11	0.06	0.13	0.02	0.03	0.02	CBCAA
Meta-cresol purple	0.80	0.69	0.94	0.40	0.34	0.00	PNSH(
Bromophenol blue	0.38	0.25	0.67	0.06	0.07	0.07	HENB
Bromothymol blue	0.64	0.43	0.95	0.17	0.19	0.12	MISD(
Thymol blue	0.95	0.79	0.95	0.43	0.42	0.14	SSPJJ
Methyl red	0.55	0.44	0.72	0.34	0.26	0.16	KIOG
Phenolphtalein	1.00	1.00	1.00	1.00	1.00	0.83	TTTT1
Phenol red	0.03	0.02	0.03	0.01	0.00	0.00	AAAA

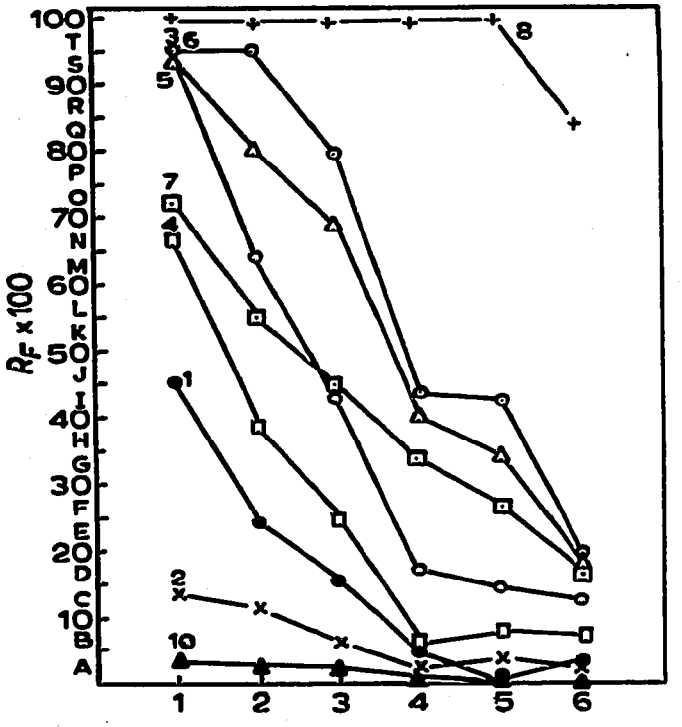

Fig. 2. R_F values of indicators plotted against the type of thin layer. Each line joins R_F values of the same indicator. 1 = Bromocresol purple, 2 = *ortho*-cresol red, 3 = *meta*-cresol purple, 4 = bromophenol blue, 5 = bromothymol blue, 6 = thymol blue, 7 = methyl red, 8 = phenolphthalein, 9 = phenol red. The layers from left to right are: 1 = Acetylated cellulose AC-10 MN, 2 = cellulose (Carlo Erba), 3 = cellulose MN, 4 = DEAE cellulose MN, 5 = aluminium oxide (Carlo Erba), 6 = silica gel (Eastman).

examined each has another "word" although several would prove non-specific in a larger range of compounds (phenol red and phenolphthalein for example).

We feel that the procedure shows enough promise that we plan to examine a few larger groups of compounds where a better chromatographic characterisation would be desirable.

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2 K. MACEK, private communication.

Received September 13th, 1971