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APPLICATION OF R_F CORRECTION IN THIN-LAYER CHROMATOGRAPHY BY MEANS OF TWO REFERENCE R_F VALUES

III. RESULTS OBTAINED IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

J. H. DHONT and C. VINKENBORG
Central Institute for Nutrition and Food Research TNO, Zeist (The Netherlands)
H. COMPAAN and F. J. RITTER
Central Laboratory TNO, Delft (The Netherlands)
A. VERWEIJ
Chemical Laboratory of the National Defence Research Organisation TNO, Rijswijk Z.H. (The Netherlands)
and
R. A. DE ZEEUW
Laboratory for Pharmaceutical and Analytical Chemistry, State University, Groningen (The Netherlands)

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SUMMARY

An interlaboratory experiment is described which proves that the method of Galanos and Kapoulas for the determination of R_F values is applicable to reversed-phase thin-layer systems.

Differences in the technique of application of the stationary phase resulted in large differences in R_F values. However, correcting the R_F values by using the method of Galanos and Kapoulas resulted in highly stabilized R_F^c values.

INTRODUCTION

In previous papers in this series[1,2], we reported on the application of the R_F correction method of Galanos and Kapoulas[3] to a non-polar single component and to a polar multicomponent solvent system. In this paper, we report results obtained in a reversed-phase system. The experiment was designed by members of the TNO Discussion Group mentioned in the previous papers.

EXPERIMENTAL

Each of the participants obtained three sealed ampoules (A, B and C). Ampoule A contained 0.1% of Oil Soluble Green, ampoule B 0.05% of Waxolin purple and 0.05% of Sudan Red G and ampoule C 0.05% of Sudan Orange R. All components were dissolved in a 1:1 mixture of acetone and benzene.

Stationary phase

Paraffin oil was used as the stationary phase. This phase was not specified more precisely, and all members used the paraffin oil available in their respective laboratories.

Mobile phase

The mobile phase was acetone–water–ammonia (sp. gr. 0.91) (7:3:1).

Impregnation techniques

The participants were asked to impregnate the thin layer with paraffin oil in one of the following ways:

(I) The plate, covered with the thin layer (silica gel, Kieselguhr), is placed in a chromatographic tank containing a 3–6% solution of paraffin oil in light petroleum. The solution is allowed to rise to a height of at least 15 cm into the thin layer. The plate is ready for use when the light petroleum has evaporated from the layer, leaving a thin film of paraffin oil on the plate.

(II) The plate covered with the thin layer is soaked in a solution of 3–6 g of

TABLE I

CHROMATOGRAPHIC CONDITIONS AS REPORTED BY THE PARTICIPANTS

Experiment No.	*Layer*	*Method of impregnation*[*]	*Amount of stationary phase in solution or suspension (g/100 ml)*
1	Kieselguhr	III	1.25
2	Silica gel	I	5
3	Silica gel	III	2.5
4	Silica gel	I	4
5	Silica gel	I	4
6	Silica gel	II	4
7	Silica gel	II	4
8	Silica gel	I	5
9	Silica gel	I	5
10	Silica gel	I	5
11	Silica gel	II	4.6
12	Silica gel	II	4.6
13	Kieselguhr	I	4.6
14	Kieselguhr	I	4.6
15	Kieselguhr	II	4.6
16	Kieselguhr	II	4.5
17	Silica gel	III	2.5
18	Silica gel	III	2.5
19	Silica gel	I	4.6
20	Silica gel	I	4.6
21	Kieselguhr	I	6
22	Kieselguhr	II	6
23	Kieselguhr	I	6
24	Kieselguhr	II	6

[*] See text.

paraffin oil in 100 ml of light petroleum. The plate is ready for use after evaporation of the solvent.

(III) A 20-g amount of Kieselguhr G or silica gel is suspended in 80 ml of a 1% solution of poly(vinyl chloride) in methyl ethyl ketone. A known amount of paraffin oil (1–3 g) is added to the suspension.

Other conditions

The participants were asked to spot the dye solutions 1.5 cm from the bottom edge of the plate. The front line was to be drawn 10 cm above the start and the plate removed from the tank as soon as the solvent front reached this line.

Some of the chromatograms were developed twice in the solvent mixture. The resulting ${}^2R_F$ values were converted into ${}^1R_F$ values by using the equation $R_F = 1 - \sqrt[n]{1 - {}^nR_F}$. These values were then used to calculate R_F values.

RESULTS AND DISCUSSION

Seven laboratories took part and all of them carried out more than one experiment under different conditions. In all, 24 independent experiments were performed.

The experimental details are summarized in Table I, and Table II gives the results reported by the participants. In the tables the components are indicated as follows:

TABLE II

R_F VALUES FOUND BY PARTICIPANTS

Experiment No.	*Components*					*Number of developments*
	Blue	*Yellow*	*Purple*	*Red*	*Orange*	
1	0.061	0.221	0.217	0.395	0.326	1
2	0.32	0.64	0.56	0.85	0.76	1
3	0.08	0.28	0.19	0.41	0.35	1
4	0.437	0.672	0.639	0.923	0.826	1
5	0.138	0.509	0.438	0.770	0.699	1
6	0.175	0.619	0.572	0.877	0.784	1
7	0.137	0.441	0.340	0.702	0.605	1
8	0.15	0.41	0.35	0.595	0.515	1
9	0.145	0.385	0.33	0.585	0.47	1
10	0.17	0.42	0.365	0.645	0.54	1
11	0.085	0.305	0.255	0.540	0.445	1
12	0.19	0.52	0.45	0.82	0.72	2
13	0.11	0.43	0.42	0.77	0.585	1
14	0.17	0.51	0.49	0.825	0.65	2
15	0.10	0.40	0.38	0.74	0.555	1
16	0.195	0.58	0.545	0.86	0.72	2
17	0.055	0.305	0.17	0.46	0.36	1
18	0.09	0.41	0.25	0.575	0.47	2
19	0.10	0.335	0.27	0.56	0.482	1
20	0.15	0.41	0.35	0.645	0.555	2
21	0.115	0.30	0.26	0.54	0.40	1
22	0.145	0.34	0.30	0.57	0.44	1
23	0.165	0.44	0.395	0.72	0.575	1
24	0.17	0.46	0.415	0.74	0.60	1

TABLE III

R_F^c VALUES

Experiment No.	*Components*			*Experiment No.*	*Components*		
	Yellow	*Purple*	*Orange*		*Yellow*	*Purple*	*Orange*
1	0.221	0.217	0.326	13	0.218	0.212	0.295
2	0.236	0.213	0.339	14	0.233	0.233	0.305
3	0.262	0.171	0.333	15	0.218	0.207	0.299
4	0.216	0.192	0.321	16	0.255	0.237	0.325
5	0.257	0.220	0.358	17	0.260	0.157	0.313
6	0.272	0.250	0.351	18	0.282	0.172	0.324
7	0.232	0.172	0.329	19	0.232	0.208	0.341
8	0.255	0.210	0.335	20	0.237	0.196	0.335
9	0.242	0.201	0.307	21	0.207	0.175	0.285
10	0.236	0.197	0.320	22	0.215	0.183	0.292
11	0.224	0.188	0.328	23	0.226	0.212	0.308
12	0.236	0.199	0.342	24	0.233	0.206	0.315

TABLE IV

MEAN R_F VALUES $\overline{R_F^c}$ VALUES AND STANDARD DEVIATIONS

Component	$\overline{R_F}$	*Standard deviation*	$\overline{R_F^c}$	*Standard deviation*
Blue	0.15	0.081	0.061	—
Yellow	0.43	0.114	0.24	0.014
Purple	0.38	0.120	0.20	0.022
Red	0.67	0.145	0.40	—
Orange	0.56	0.126	0.32	0.017

Yellow and Blue: components of Oil Soluble Green
Purple: component of Waxolin Purple
Red: Sudan Red G
Orange: Sudan Orange R

The R_F^c values were calculated as described in our previous papers[1,2], with the R_F values of Blue and Red as the R_F^0 values. The R_F^c values are collected in Table III and the mean values and standard deviations are given in Table IV. Table IV also reveals a substantial improvement in reproducibility in spite of the widely different conditions used in the separate experiments. Similar results were obtained in systems using a non-polar single-component solvent and a polar multicomponent solvent[1,2].

The results reported in this series of papers clearly show that the Galanos and Kapoulas method[3] for calculating R_F is also very reliable in thin-layer chromatography. Accordingly, compilations of R_F values, *e.g.*, in the *Journal of Chromatography*, can be of great value, as the only condition to be satisfied for reproduction of the tabulated values is the availability of two different R_F values that can be used as R_F^0 values. These two reference compounds can be chosen arbitrarily.

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