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

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SUMMARY

The interlaboratory experiment described in this paper proves that, for the system investigated, the method of R_F corrections originally proposed by GALANOS AND KAPOULAS for paper chromatography is likewise applicable to thin-layer chromatography.

Highly reliable results with five non-structurally related test compounds were obtained under a wide variety of experimental conditions. The collaborative investigation will be continued, with other compounds and other thin-layer chromatographic systems, to see whether the method implies a wider applicability.

INTRODUCTION

Since the advent of thin-layer chromatography, occasionally suggestions for standardizing R_F value determinations which often appeared to be poorly reproducible have been published. Some of the recommendations were tested[1,2] and the method of GALANOS AND KAPOULAS[3]—described for paper chromatography—appeared to have the greatest applicability. Generally this method, published in 1964 and mentioned in PATAKI's book[4], received little attention. WILLIAMS, however, came to a similar expression of the correction equation by using statistical techniques[5]. PRINZLER AND

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* Report of a study group on "R_F correction methods in TLC". Correspondence should be directed to the first author.

TAUCHMANN[6] investigated the behaviour of organic sulphoxides on thin-layer plates of alumina which were equilibrated in atmospheres of different relative humidity. They compared R_F with R_{st} values (R_F values calculated with reference to one standard substance) and with $R_F{}^c$ values (R_F values corrected according to the procedure of GALANOS AND KAPOULAS). Independent of the relative humidity, only the $R_F{}^c$ values proved to be reproducible.

The subject has recently been discussed by the TNO Discussion Group on Chromatography*, and some of its members decided to participate in a comparative interlaboratory investigation of the method of GALANOS AND KAPOULAS. A study group on "R_F correction methods in TLC" was thus assigned to work out the data obtained. This paper presents the results of the investigation.

TABLE I

DETAILS OF THE THIN-LAYER CHROMATOGRAPHIC EXPERIMENTS RECORDED BY THE PARTICIPANTS

Expt. No.	Silica gel used	Activation	Chamber saturation[a]
I	Silica Gel G, Merck	not activated	+
II	Silica Gel G, Merck	115°, 1 h	+
III	Camag, DSF-5	not activated	+
IV	Silica Gel G, F 254, Merck	110°, 45 min	—
V	Silica Gel, Camag DO	290°, 30 min	+
VI	Silica Gel G, Merck	110°, 30 min	+
VII	Silica Gel G, Merck	110°, 30 min	—
VIII	Silica Gel G, Merck	not activated	+
IX	Silica Gel G, Merck	not activated	—
X	Fertigplatte, F 245, Merck	110°, 30 min	+
XI	Fertigplatte, F 245, Merck	110°, 30 min	—
XII	Eastman sheet	not activated	+
XIII	Eastman sheet	not activated	—
XIV	Silica Gel G, Merck	110°, 1 h	+
XV	Silica Gel H, Fluka	120°, 17 h	+
XVI	Silica Gel G, Merck	110°, 1 h	—
XVII	Silica Gel G, Merck	not activated	+
XVIII	Silica Gel H, Merck	115°, 1 h	+
XIX	Silica Gel G, Merck	100°, 1 h	+
XX	Silica Gel G, Merck	100°, 1 h	+
XXI	Silica Gel G, Merck	100°, 1 h	+
XXII	Silica Gel G, Merck	100°, 1 h	—
XXIII	Silica Gel G, Merck	100°, 1 h	—
XXIV	Silica Gel G, Merck	100°, 1 h	—
XXV	Silica Gel G, Merck	105°, 1 h	+
XXVI	Silica Gel G, Merck	110°, 3 h	—
XXVII	Silica Gel H, Merck	110°, 3 h	—
XXVIII	Silica Gel HR, Merck	110°, 3 h	—
XXIX	Silica Gel HF, Merck	110°, 3 h	—
XXX	MN-folygram	110°, 3 h	—
XXXI	DC-Fertigplatte, F 245, Merck	not activated	—
XXXII	Silica Gel, Woelm	110°, 3 h	—
XXXIII	DC-Fertigplatte, F 254, Merck	not activated	S-chamber
XXXIV	Eastman sheet, F 254	not activated	S-chamber
XXXV	DC-Fertigplatte, F 254, Merck	not activated	+
XXXVI	Silica gel, F 254, Alufolie, Merck	not activated	+
XXXVII	Silica Gel G, Merck	110°, 1 h	+

[a] + = saturated; — = non-saturated.

* This is an informal discussion group in The Netherlands.

EXPERIMENTAL

Each of the participants had obtained two sealed ampoules, marked 1 and 2. Ampoule 1 contained the test mixture of STAHL as delivered by Desaga (Heidelberg, G.F.R.). The components of the mixture are indicated by Y (yellow), B (blue) and R (red). Ampoule 2 contained a mixture of the 2,4-dinitrophenylhydrazones of methanal and *n*-dodecanal dissolved in ethyl acetate (concn. 2.5 mg/ml); they are indicated by C_1 and C_{12}, respectively. The following chromatographic system was prescribed: a layer of silica gel; benzene as solvent; application 1.5 cm from the bottom of the plate; a 10-cm length of run; and a 3-μl sample. No further conditions had been given.

TABLE II

R_F VALUES FOUND

Expt. No.	Components[a]				
	Y	R	B	C_1	C_{12}
I	0.467	0.207	0.132	0.342	0.590
II	0.510	0.207	0.085	0.369	0.646
III	0.372	0.167	0.100	0.260	0.482
IV	0.625	0.235	0.075	0.445	0.795
V	0.340	0.140	0.070	0.270	0.450
VI	0.445	0.225	0.105	0.360	0.555
VII	0.680	0.300	0.095	0.535	0.845
VIII	0.385	0.165	0.080	0.320	0.520
IX	0.750	0.360	0.170	0.590	0.880
X	0.355	0.140	0.060	0.280	0.450
XI	0.700	0.325	0.140	0.550	0.795
XII	0.540	0.295	0.190	0.435	0.615
XIII	0.935	0.660	0.410	0.855	0.955
XIV	0.44	0.18	0.07	0.36	0.59
XV	0.67	0.23	0.12	0.51	0.84
XVI	0.55	0.17	0.047	0.46	0.79
XVII	0.47	0.20	0.09	0.40	0.63
XVIII	0.42	0.165	0.085	0.30	0.54
XIX	0.39	0.14	0.05	0.25	0.52
XX	0.39	0.13	0.04	0.25	0.55
XXI	0.50	0.19	0.08	0.38	0.68
XXII	0.65	0.25	0.11	0.42	0.80
XXIII	0.58	0.18	0.05	0.37	0.79
XXIV	0.64	0.26	0.12	0.43	0.80
XXV	0.71	0.44	0.25	0.56	0.80
XXVI	0.67	0.24	0.11	0.49	0.82
XXVII	0.60	0.21	0.09	0.43	0.78
XXVIII	0.59	0.21	0.08	0.45	0.83
XXIX	0.67	0.24	0.11	0.47	0.82
XXX	0.77	0.31	0.12	0.57	0.90
XXXI	0.65	0.22	0.10	0.48	0.79
XXXII	0.56	0.17	0.03	0.38	0.82
XXXIII	0.61	0.23	0.12	0.45	0.73
XXXIV	0.80	0.44	0.36	0.68	0.88
XXXV	0.25	0.10	0.04	0.185	0.34
XXXVI	0.33	0.12	0.05	0.24	0.43
XXXVII	0.48	0.18	0.07	0.39	0.64

[a] Y, R and B refer to the yellow, red and blue components of STAHL's test mixture. C_1 and C_{12} are 2,4-dinitrophenylhydrazones of methanal and *n*-dodecanal.

The participants were free to saturate the tank with benzene or to use unsaturated tanks, to use either activated or non-activated plates, and to choose the carrier material (homemade layers, plastic sheets, prefabricated plates).

Twelve laboratories participated in the investigation. Some of them carried out more than one experiment; only those carried out under essentially different conditions (employing different kinds of silica gel and/or chamber (un)saturation) were then accepted as independent experiments. Thirty-seven independent experiments were thus obtained. Table I gives experimental details recorded by the participants.

RESULTS AND DISCUSSION

Table II shows the R_F values found by the participants. A chromatogram developed in one of the participating laboratories[1] was taken as the standard. The R_F values derived from this chromatogram are designated R_F^0 (Table II, Expt. No. I). The correction formula proposed by GALANOS AND KAPOULAS has the form

$$R_F^c = aR_F + b$$

where R_F^c stands for the corrected R_F value. For the standard chromatogram R_F^c and R_F^0 are, of course, identical. Constants a en b are derived from two reference R_F values from the standard chromatogram (R_F^0) and similar reference R_F values of these compounds from the chromatogram that is to be corrected.

$$a = \frac{R_F^0(x) - R_F^0(y)}{R_F(x) - R_F(y)} \qquad b = R_F^0(x) - aR_F(x)$$

where x and y refer to the reference compounds, x and y.

An example of the calculation is given below. The R_F values of Y and B were used as the reference values for the calculation of a and b. Next, the R_F values of R, C_1 and C_{12} were corrected with the aid of the correction formula. Additionally, the R_F values of C_{12} and R were used to calculate a and b in order to correct the R_F values of Y, C_1 and B. Thus, for each chromatogram, two R_F^c values for C_1 were obtained. Both corrections could be checked, since C_1 should give essentially the same R_F^c value irrespective of the reference values used. Table III records the mean R_F^c value for C_1.

From I   From II

(Standard chromatogram, Table II)   (Chromatogram to be corrected, Table II)

R_F^0 of Y = 0.467		R_F of Y = 0.510
R_F^0 of B = 0.132		R_F of B = 0.085
Difference = 0.335		Difference = 0.425
$a = \frac{0.335}{0.425} = 0.790$		$b = 0.467 - 0.790 \times 0.510 = 0.065$

Using these values for a and b, the R_F values of Expt. No. II are now corrected:

R_F^c of R   = $0.790 \times 0.207 + 0.065 = 0.228$

R_F^c of C_1 = $0.790 \times 0.369 + 0.065 = 0.356$

R_F^c of C_{12} = $0.790 \times 0.646 + 0.065 = 0.575$

Exactly the same procedure was followed using R_F^0 values of C_{12} and R from chromatogram I and the corresponding R_F values of these compounds from chromatogram II. The two R_F^c values found for C_1 are 0.356 and 0.349. These two values obtained were always similar.

TABLE III

CORRECTED R_F VALUES (R_F^c) DERIVED FROM TABLE II

Expt. No.	Components[a]				
	Y	R	B	C_1	C_{12}
I	0.467	0.207	0.132	0.342	0.590
II	0.471	0.228	0.101	0.352	0.575
III	0.462	0.215	0.140	0.330	0.602
IV	0.480	0.229	0.104	0.357	0.571
V	0.454	0.239	0.120	0.380	0.603
VI	0.461	0.250	0.105	0.383	0.575
VII	0.474	0.250	0.063	0.379	0.562
VIII	0.446	0.226	0.117	0.386	0.616
IX	0.494	0.239	0.067	0.374	0.541
X	0.484	0.220	0.120	0.386	0.574
XI	0.513	0.239	0.066	0.386	0.525
XII	0.501	0.233	0.081	0.371	0.538
XIII[b]	—	—	—	—	—
XIV	0.450	0.231	0.104	0.384	0.603
XV	0.483	0.200	0.138	0.376	0.572
XVI	0.443	0.214	0.132	0.391	0.627
XVII	0.447	0.228	0.109	0.395	0.608
XVIII	0.470	0.212	0.129	0.347	0.587
XIX	0.459	0.221	0.126	0.328	0.595
XX	0.444	0.217	0.125	0.325	0.620
XXI	0.449	0.220	0.120	0.363	0.611
XXII	0.485	0.218	0.124	0.325	0.560
XXIII	0.458	0.215	0.125	0.330	0.597
XXIV	0.476	0.221	0.108	0.329	0.569
XXV	0.495	0.263	0.060	0.346	0.533
XXVI	0.495	0.209	0.121	0.359	0.557
XXVII	0.465	0.211	0.089	0.405	0.586
XXVIII	0.441	0.217	0.126	0.365	0.624
XXIX	0.491	0.210	0.078	0.353	0.557
XXX	0.505	0.230	0.083	0.392	0.534
XXXI	0.496	0.205	0.127	0.373	0.553
XXXII	0.437	0.219	0.124	0.341	0.630
XXXIII	0.498	0.207	0.122	0.366	0.550
XXXIV	0.521	0.193	0.139	0.386	0.528
XXXV	0.447	0.227	0.111	0.352	0.610
XXXVI	0.456	0.211	0.110	0.349	0.582
XXXVII	0.457	0.222	0.115	0.387	0.598

[a] Abbreviations, see Table II.
[b] For explanation see text.

The R_F^c values of Y, R, B, C_1 (the mean value only) and C_{12} from all experiments are shown in Table III. For uniformity and statistical testing, all values in this table were calculated to three decimal places.

Because of the high R_F value of C_{12} on the chromatogram from Expt. XIII (Table II), its R_F^c values are not recorded in Table III. Frequently it has been found that R_F values close to 1.00 cannot be used to calculate R_M values[7,8]. For the calculations of the R_F^c values, using the correction procedure of GALANOS AND KAPOULAS a similar result was obtained.

Table IV summarizes the means and the standard deviations of the populations

TABLE IV

STATISTICS CALCULATED FROM TABLES II AND III

Compound[a]	From Table II		From Table III	
	Mean R_F	s[b]	Mean R_F[c]	s[b]
Y	0.554	0.151	0.473	0.026
R	0.233	0.105	0.223	0.010
B	0.114	0.078	0.110	0.022
C_1	0.431	0.113	0 364	0.020
C_{12}	0.694	0.156	0.579	0.030

[a] Abbreviations, see Table II.
[b] Standard deviation for a single observation: $s = \sqrt{\Sigma \frac{(x - \bar{x})^2}{n - 1}}$

recorded in Tables II and III. It appears that, using the method of GALANOS AND KAPOULAS, an extraordinary gain in reproducibility has been obtained.

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