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## $R_F$ correction in thin-layer chromatography

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(First received April 21st, 1978; revised manuscript received October 9th, 1978)

Experimental $R_F$ values appear to be poorly reproducible because they depend on many experimental variables that cannot be controlled easily. As a result, methods have been suggested for applying corrections to $R_F$ values in order to make them generally interchangeable, such corrections relating values of different origin to some arbitrary reference.

Dhont and De Rooy[1] advocated the use of the ratio of the $R_F$ value of a compound to the $R_F$ value of a reference:

$$R_F = aR_{F,\text{ref}}$$

Although this method is frequently used, the potential of this single reference correction is very limited, as was pointed out by Geiss[2].

Surprisingly reproducible $R_F$ values were obtained by Galanos and Kapoulas[3] using a correction with two reference substances. This method, described in the first instance for paper chromatography, was later tested in thin-layer chromatography by Dhont et al.[4,5] in a large-scale inter-laboratory experiment. They found a large increase in reproducibility of $R_F$ values with both a single- and a multi-component mobile phase.

In this paper, we present an algorithm derived from thermodynamic and chromatographic principles for the correction of $R_F$ values, also using two reference compounds. It is demonstrated that this algorithm is more powerful than the Galanos and Kapoulas correction.

## THEORETICAL

In thin-layer chromatography, the position of a spot after development is characterized by its $R_F$ value, which is the migration distance of the spot divided by the migration distance of the solvent front.

However, the distribution of a substance between the mobile and the stationary phases is not directly related to $R_F$ but to $R$, the ratio of the volume of the mobile phase between start and the spot to the volume between the start and the front. The amount of mobile phase is proportional to distance only at $R_F$ values below

0.8. Only below such $R_F$ values is the relationship between $R$ and $R_F$ linear, and a multiplication factor $\xi$ is needed[2] in order to convert $R$ into $R_F$:

$$R = \xi R_F$$

($\xi \approx 1.1$–$1.4$). A quantity that is comparable to a partition coefficient is $R_M$, defined by

$$R_M = \log \left( \frac{1}{R} - 1 \right)$$

It is known that a change in some partitioning system will often result in the addition of the same increment to the partition coefficients of many substances[6]. We might therefore expect that the same would hold true for chromatographic systems\*. In other words, for many substances $R_M$ in system $i$ ($R_{Mi}$) will equal $R_M$ in system $j$ ($R_{Mj}$) plus some constant that depends only on $i$ and $j$:

$$R_{Mi} = R_{Mj} + \log C_{i,j}$$

This is in accordance with Brenner et al.[7], who stated that the difference in the $R_M$ values of two compounds is not sensitive to variations in the experimental conditions.

Fundamentally, this relationship holds only in partition chromatography. However, Ościk[8] showed theoretically that the same relationship can also be applied in adsorption chromatography, albeit with some limitations.

We have now introduced two factors, $\xi$ and $C_{i,j}$, which are susceptible to experimental variations, leading to the equation

$$\log \left( \frac{1}{\xi_i R_{Fi}} - 1 \right) = \log \left( \frac{1}{\xi_j R_{Fj}} - 1 \right) + \log C_{i,j}$$

Taking antilogarithms and rearranging, we obtain

$$\frac{1}{R_{Fi}} = \frac{\xi_i C_{i,j}}{\xi_j} \cdot \frac{1}{R_{Fj}} + \xi_i (1 - C_{i,j})$$

Considering only the standard system $i$ and some other system $j$, the coefficients $\xi_i C_{i,j}/\xi_j$ and $\xi_i(1 - C_{i,j})$ are constants for that particular combination, giving

$$\frac{1}{R_{Fi}} = \frac{a}{R_{Fj}} + b \tag{1}$$

To correct $R_F$ values found with system $j$, so that they will correspond to those found with system $i$, one has to determine the value of the constants $a$ and $b$ from the $R_F$ values of two reference substances 1 and 2. These $R_F$ values satisfy the equations

$$\frac{1}{R_{Fi,k}} = \frac{a}{R_{Fj,k}} + b$$

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\* By "chromatographic system", or "system", we denote the assembly of mobile phase, thin layer and all conditions pertinent to one actual plate. Hence, two plates are two systems even when all characteristics are nominally the same.

with $k = 1$ or 2. From these equations, $a$ and $b$ are calculated:

$$a = (1/R_{Fi,1} - 1/R_{Fi,2})/(1/R_{Fj,1} - 1/R_{Fj,2})$$
$$b = 1/R_{Fi,1} - a/R_{Fj,1}$$

The algorithm introduced by Galanos and Kapoulas[3] is given by

$$R_{Fi} = aR_{Fj} + b \tag{2}$$

The use of this algorithm is similar to that of the algorithm derived in this paper.

EXPERIMENTAL

To explore the scope and limitations of the correction equation derived, two different experiments were selected as outlined below.

*The same mobile phase on different plates*

Experimental results reported by Dhont et al.[4,5] were used. In these experiments, the different test substances were not structurally related.

*Different mobile phases on one kind of plate*

Nine different steroids were spotted on silica plates (Fertigplatte, Merck, Darmstadt, G.F.R.) 2 cm from the lower edge. The plates were placed in saturated chambers and developed over a length of 15 cm. The mobile phases used were mixtures of *n*-heptane and acetone in various proportions. After chromatography the spots were revealed by spraying with sulphuric acid (2% in ethanol) and heating to 110°.

RESULTS AND DISCUSSION

*Correction of the data obtained by Dhont et al.[4] with a single-component mobile phase*

Table I summarizes the results of the correction procedure. The notation and abbreviations of Dhont et al.[4] are used throughout. The reference substances were chosen according to Dhont et al., *i.e.*, $C_{12}$ is the substance with the highest $R_F$ value and R is the substance with the lowest $R_F$ value, which on most plates is still above 0.1. Contrary to the study of Dhont et al., plate XIII was included, making a total of 37 plates used in the correction procedure.

From Table I it can be concluded that the proposed correction gives a greater reduction in the variance than the correction of Galanos and Kapoulas[3]. With two of the three substances this reduction is significant at the 5% level. Moreover, the Galanos and Kapoulas procedure, when applied to plate XIII of Dhont et al., gives poor results, whereas the new algorithm gives acceptable values. This can be made clearer by plotting the $R_F$ values on plate XIII against those on the standard plate. as shown in Fig. 1.

The curved line in Fig. 1, representing the correction proposed in this paper fits the experimental points better than the straight line representing the Galanos and Kapoulas correction.

TABLE I

SUMMARY OF RESULTS ($R_F \times 1000$) OBTAINED WITH A SINGLE-COMPONENT MOBILE PHASE

| Parameter | Substance* | | | | |
|---|---|---|---|---|---|
| | Y | R | B | $C_1$ | $C_{12}$ |
| Standard plate | 467 | 207 | 132 | 342 | 590 |
| Raw data: | | | | | |
| Mean | 554 | 233 | 111 | 419 | 694 |
| S.d. | 153 | 107 | 80 | 134 | 158 |
| Plate XIII | 935 | 660 | 410 | 855 | 955 |
| Correction (G–K**): | | | | | |
| Mean | 474 | 207 | 102 | 362 | 590 |
| S.d. | 27 | — | 46 | 29 | — |
| Plate XIII | 564 | 207 | −118 | 460 | 590 |
| Correction (this work): | | | | | |
| Mean | 482 | 207 | 94 | 371 | 590 |
| S.d. | 19 | — | 21 | 26 | — |
| Plate XIII | 542 | 207 | 91 | 398 | 590 |

* See Dhont et al.[4] for abbreviations.
** G–K = Galanos and Kapoulas[3].
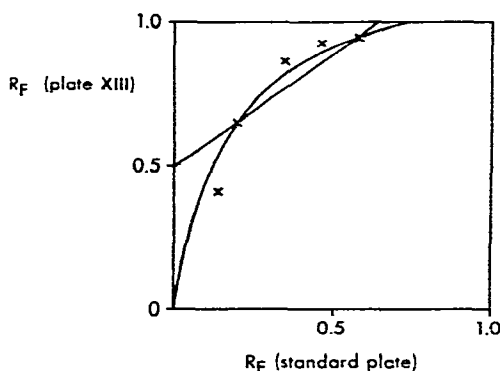


Fig. 1. Relationship between $R_F$ values on plate XIII and the standard plate.

Curved correction graphs have also been reported by Moffat[9], who, in his correction procedure, approximated these curved lines by some straight-line segments. Clearly, this compelled him to use more than two reference substances.

*Correction of the data obtained by Dhont et al.[5] with a polar multi-component mobile phase*

In this experiment 49 plates were used. Dhont et al.[5] left two sets of four plates each out of their correction procedure, as they gave unexplained deviations. Again, for notations and abbreviations the reader is referred to the original paper of Dhont et al.[5]. The substances denoted by C and D were used as reference substances.

The results in Table II indicate that the new correction is superior to that of Galanos and Kapoulas[3]; in two of the three cases the difference is significant.

TABLE II

SUMMARY OF RESULTS ($R_F \times 1000$) OBTAINED WITH A POLAR MULTI-COMPONENT MOBILE PHASE

| Parameter | Substance* | | | | |
|---|---|---|---|---|---|
| | T | G | D | N | C |
| Standard plate | 470 | 140 | 790 | 340 | 90 |
| Raw data: | | | | | |
| Mean | 571 | 201 | 881 | 444 | 156 |
| S.d. | 91 | 55 | 70 | 88 | 45 |
| Correction (G–K**): | | | | | |
| Mean | 492 | 133 | 790 | 369 | 90 |
| S.d. | 65 | 31 | — | 55 | — |
| Correction (this work): | | | | | |
| Mean | 425 | 120 | 790 | 304 | 90 |
| S.d. | 55 | 24 | — | 34 | — |

* See Dhont et al.[5] for abbreviations.
** G–K = Galanos and Kapoulas[3].

## Correction of data obtained with mobile phases consisting of two solvents mixed in several proportions

In practical thin-layer chromatography, an inadequately controlled variable is the exact composition of the eluent. In this experiment, this factor has been greatly exaggerated. However, the proposed correction procedure should work well when all substances undergo the same shift in $R_M$ values, as has been previously reported for groups of related substances, for instance by Biagi and co-workers[10,11] and Howe[12].

The results of the correction procedure are summarized in Table III, and

TABLE III

SUMMARY OF RESULTS ($R_F \times 1000$) OBTAINED WITH TWO SOLVENTS MIXED IN SEVERAL PROPORTIONS

| Parameter | Substance* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Standard plate (plate XI) | 540 | 480 | 400 | 430 | 450 | 330 | 310 | 220 | 190 |
| Raw data: | | | | | | | | | |
| Mean | 661 | 614 | 568 | 574 | 563 | 489 | 463 | 381 | 349 |
| S.d. | 169 | 193 | 192 | 203 | 186 | 231 | 229 | 218 | 209 |
| Correction (G–K**) | | | | | | | | | |
| Mean | 540 | 489 | 442 | 444 | 427 | 353 | 323 | 228 | 190 |
| S.d. | — | 26 | 62 | 32 | 27 | 53 | 45 | 21 | — |
| Correction (this work): | | | | | | | | | |
| Mean | 540 | 486 | 430 | 435 | 412 | 338 | 306 | 219 | 190 |
| S.d. | — | 18 | 71 | 20 | 51 | 13 | 9 | 12 | — |

* 1 = Lynestrenol; 2 = mestranol; 3 = testosterone acetate; 4 = estrone; 5 = cis-andros-terone; 6 = hydrocortisone acetate; 7 = prednisolone acetate; 8 = hydrocortisone; 9 = prednisolone.
** G–K = Galanos and Kapoulas[3].

details of the $R_F$ values are given in Table IV. In this experiment, plate XI was chosen as the standard system and substances 1 and 9 served as references. The number of plates used was 16.

The results indicate that the variation in $R_F$ values caused by substantial changes in the composition of the mobile phase can be reduced to acceptable levels by using the new correction procedure. The correction proposed in this paper is significantly better in four instances, while the Galanos and Kapoulas correction is better in only one instance.

TABLE IV

$R_F \times 1000$ VALUES FOUND WITH TWO-COMPONENT MOBILE PHASES WITH VARIOUS MIXING RATIOS

| Plate | Proportions in mobile phase (v/v) | | Substance* | | | | | | | | |
|-------|---------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Heptane | Acetone | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| I | 0 | 100 | 800 | 780 | 760 | 760 | 720 | 740 | 720 | 660 | 620 |
| II | 0 | 100 | 820 | 810 | 780 | 790 | 750 | 760 | 740 | 680 | 650 |
| III | 10 | 90 | 820 | 800 | 770 | 770 | 740 | 730 | 690 | 610 | 580 |
| IV | 10 | 90 | 810 | 800 | 770 | 760 | 740 | 720 | 700 | 620 | 580 |
| V | 20 | 80 | 800 | 740 | 730 | 710 | 690 | 650 | 640 | 530 | 480 |
| VI | 20 | 80 | 770 | 740 | 720 | 710 | 670 | 650 | 610 | 500 | 480 |
| VII | 30 | 70 | 760 | 740 | 750 | 700 | 690 | 600 | 550 | 460 | 400 |
| VIII | 30 | 70 | 720 | 690 | 680 | 660 | 630 | 540 | 530 | 420 | 400 |
| IX | 40 | 60 | 740 | 690 | 480 | 640 | 640 | 540 | 500 | 390 | 350 |
| X | 40 | 60 | 780 | 710 | 490 | 670 | 660 | 560 | 520 | 410 | 350 |
| XI | 50 | 50 | 540 | 480 | 400 | 430 | 450 | 330 | 310 | 220 | 190 |
| XII | 50 | 50 | 540 | 490 | 400 | 440 | 460 | 330 | 310 | 220 | 200 |
| XIII | 60 | 40 | 470 | 400 | 400 | 360 | 370 | 230 | 200 | 150 | 120 |
| XIV | 60 | 40 | 470 | 390 | 400 | 350 | 350 | 230 | 200 | 140 | 110 |
| XV | 70 | 30 | 380 | 280 | 280 | 220 | 220 | 110 | 90 | 50 | 40 |
| XVI | 70 | 30 | 380 | 280 | 280 | 210 | 230 | 100 | 90 | 40 | 40 |

* As in Table III.

CONCLUSION

In the past 15 years, suggestions have been made for improving the reproducibility and exchangeability of experimental $R_F$ values. In this respect, the empirical correction procedure proposed by Galanos and Kapoulas[3] showed great promise. We have shown in this paper that a simple new correction formula, derived from chromatographic and thermodynamic principles, is more powerful than existing methods. Even with eluents with widely divergent compositions very good corrections can be obtained.

We expect that the use of this new algorithm will greatly enhance the significance of reporting $R_F$ values. In addition to current methods, this procedure might be helpful in the identification of compounds in forensic chemistry. It is therefore proposed that in the future experimental $R_F$ values should be reported in the literature together with the $R_F$ values of two easily available reference substances.

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