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NOTES

Multiple Development Technique in Partition Chromatography

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INTRODUCTION.

One of the difficulties of analytical procedures is the separation of two compounds having similar properties. This is the case in chromatographic techniques which resolve poorly if at all a mixture of two substances of near R*f* values. In such cases it is common practice to repeat the chromatography with the same solvent, this leading in general to an improvement of the separation. This method is known as the multiple development technique [1-17]. However it is important to know before hand how many times a chromatography has to be repeated for the best results to be attained. To avoid relying only on experience, we undertook a theoretical study of the multiple development technique, and demonstrated that a separation can be improved to an optimal point. The general formula giving the number n of successive developments needed to reach this optimal resolution is given, as well as a graph which is of more practical convenience. A serie of multiple developments with various mixtures of components have been carried out to control the conclusions attained by the theoretical approach. The results agree well with the predictions.

CHROMATOGRAPHIC BEHAVIOUR OF A MIXTURE OF TWO COMPONENTS A AND B.

We represent by a and b the R*f* of components A and B respectively, with $a < b$. Since we use the same solvent throughout, we shall assume that a and b remain unchanged during the successive developments. Suppose that the solvent migrates always the same distance. The distances covered by A after successive developments are respectively a, $a(1-a)$, $a(1-a)^2 \ldots a(1-a)^{n-1}$. Hence, the total distance of migration S_A is represented by $S_A = 1 - (1-a)^n$, the sum of a decreasing geometrical progression. The same applies for B whose migration S_B after n successive developments is $S_B = 1 - (1-b)^n$. The point of interest is the interval D between A and B : $D = S_B - S_A = (1-a)^n - (1-b)^n$. D is maximum under the conditions where its derivative D′ equals 0.

D can also be written : $D = \exp [n. \ln(1 - a)] - \exp [n. \ln(1 - b)]$

thus $D' = 1n(1 - a). \exp [n.\ln(1 - a)] - 1n(1 - b).\exp [n.\ln(1 - b)$

$D' : (1 - a)^n 1n(1 - a) - (1 - b)^n 1n(1 - b)$

$D' = O$ when $(1 - a)^n 1n(1 - a) = (1 - b)^n 1n(1 - b)$

or $$\left(\frac{1 - a}{1 - b}\right)^n = \frac{1n(1 - b)}{1n(1 - a)}$$

or $$n \log \left(\frac{1 - a}{1 - b}\right) = \log \frac{1n(1 - b)}{1n(1 - a)}$$

or $$n \frac{\log \left(\frac{1n(1 - b)}{1n(1 - a)}\right)}{\log \left(\frac{1 - a}{1 - b}\right)} = \frac{\log \left(\frac{\log(1 - b)}{\log(1 - a)}\right)}{\log \left(\frac{1 - a}{1 - b}\right)}$$

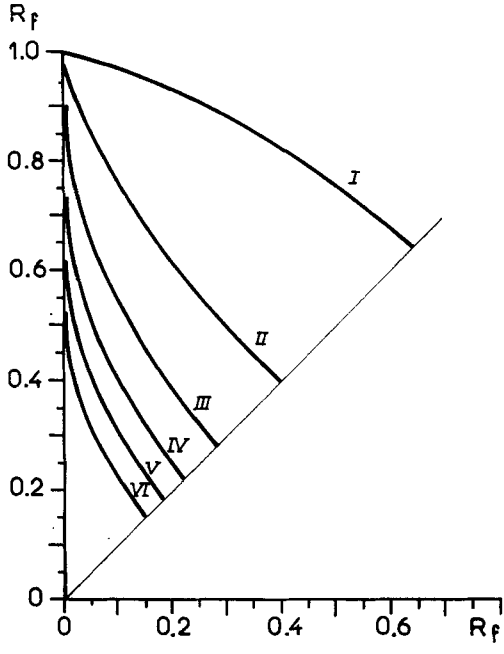

FIG. 1. Calculated values of n as a function of R_f of compounds to be separated by successive chromatographies.

abscissae : R_f of the slowest migrating compound

ordinates : R_f of the fastest migrating compound.

The value of n thus determined gives the number of successive developments needed to achieve the optimal separation of A from B. n may take integral or fractional values depending on the values for a and b. An interesting case is afforded by the situation where $1 < n < 2$, which raises the question whether a second development leads necessarily to an improvement in separation as compared with one only. To deal with this question we shall take the problem the other way round and ask whether there are conditions where a second development is not beneficial. This means conditions where the first interval D_1 equals the second D_2, or $D_1 - D_2 = (a - b)(a + b - 1) = 0$. Obviously no comment will be made if $a = b$. On the other hand, if $a + b = 1$ a second chromatographic development has no value. It is clear that several developments are of interest only if $a + b < 1$. For practical purposes, n has been calculated as a function of a and b. The integral values of n are plotted in Figure 1. The straight line of slope 1 gives the limit where no separation can be expected as it corresponds to $a = b$.

TABLE I. Comparison of calculated and experimental n values. Paper chromatography on Whatman 3 in preequilibrated tanks. Solvent : n Butanol : acetic acid : water : 90 : 15 : 33. The solvent is heated at 80° C for 1 hr and cooled to 22° before use; this treatment stabilizes the mixture. The compounds are deposed as spots (analytical) or as bands (preparative).

Substances	R_f	n calculated	n experimental	Conditions
Serine	0.199			
and		3.86	4	ascend. (analytical)
threonine	0.260			
Serine	0.181		5	descend. (analytical)
and		4.3	5,6	descend. (preparative)[a]
threonine	0.237		6-9	descend. (preparative)[b]
Threonine	0.260		3	ascend. (analytical)
and		3.0	4	ascend. (preparative)[a]
alanine	0.308		6,7	ascend. (preparative)[b]
Valine	0.400		2	descend. (analytical)
and		1.77	4	descend. (preparative)[a]
phenylalanine	0.468		4	descend. (preparative)[b]
Valine	0.523		1	ascend. (analytical)
and		1.27	3	ascend. (preparative)[a]
phenylalanine	0.623		5	ascend. (preparative)[b]

[a] band deposit of 0.5 mg per cm of starting line.

[b] band deposit of 1 mg per cm.

EXPERIMENTAL CONTROL.

In the theoretical treatment we have made the assumption that the R_f values a and b are constants throughout the successive developments. However, it is not always the case [11,18,19]. It was therefore of importance to control by experimental measurements the predictions afforded by the general equation derived above. Descending and ascending chromatographies have been run, in tanks preequilibrated with the solvents, at constant temperature. For convenience ^{14}C labeled material has been used, in various amounts, ranging from a spot of a few μg as in analytical detection, to 0,5 mg and 1 mg per cm, deposed as a band at the starting line, as wanted for preparative purposes. The intervals between the compounds submitted to the assays were measured from the autoradiographies of the chromatograms.

Table 1 corresponds to chromatographic separation on paper Whatman 3. The experimentally defined optimal number of successive chromatographies agrees well with the figure calculated from the R_f values when the amount of material spotted on the paper is small (referred as analytical). Increasing this amount to 1 mg per cm of starting line probably modifies the R_f values, and a larger number of chromatographies are required for optimal separation, than calculated. By thin layer chromatography (table II) on cellulose MN 300,

TABLE 2. Comparison of calculated and experimental *n* values. Thin layer chromatography on cellulose MN 300. Solvent : *n* Butanol : acetic acid : water : 90 : 15 : 33. Heated as in Table I. Ascending chromatography. Analytical amounts of compounds, deposed as spots.

Substances	R_f	*n* calculated	*n* experimental
Arginine and serine	0.185 – 0.207	4.6	6-8
Lysine and arginine	0.133 – 0.185	5.85	6-8
Glucose and fructose	0.24 – 0.28	3.4	4
Threonine and alanine	0.261 – 0.287	3.1	6-8
Valine and phenylalanine	0.483 – 0.550	1.4	2

the experimental data are of the same magnitude as those calculated, except in the case of the separation of threonine and alanine, which deviates by a factor 2.5. It thus appears that when a problem of separation of two compounds of close R_f values arises, the graph of Figure 1 is able to give a solution. If the value for n found is fractional, the next higher integer value has to be taken.

The technique described has found a systematic application in the preparative separation and purification of labeled compounds. The wanted derivative is often contaminated with closely related labeled substances which in a number of conditions migrate near the main compound. The multiple development technique allows a fair purification without the troublesome processes of successive elution, concentration and spotting on new sheets of paper or thin layers. Furthermore, losses and degradations due to these extra-handlings are avoided. Finally the amount of different chromatographic systems can be kept to a minimum, an important feature in a production line.

It is our experience that the multiple development technique combined with classical methods has consistently increased the yield of pure products while decreasing their cost.

E. SALA and P. FROMAGEOT

Service de Biochimie, Département de Biologie,

Centre d'Etudes Nucléaires de Saclay, BP N° 2, 91 Gif sur Yvette, France

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