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C_8 AND C_{18} REVERSED-PHASE HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY ON CHEMICALLY BONDED LAYERS FOR ENVIRONMENTAL TRACE ANALYSIS AND FOR OPTIMIZATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The outstanding properties of chemically bonded phases on high-performance thin-layer chromatographic (HPTLC) plates offer a wide range of applications in environmental analysis, not only because of simple enrichment techniques on the plates. Column optimization is another valuable field of application. The theoretical background of the latter is given in a simple treatment showing that accurate data transfer from HPTLC to high-performance liquid chromatography (HPLC) is possible, especially in reversed-phase column separations. By simultaneous multi-phase sample analysis on HP plates, optimal conditions for HPLC column separations are easily found. Two-dimensional separations on HPTLC plates show, in addition to the possibilities of on-plate controlled enrichment, that TLC is often unsurpassable in environmental trace analysis in comparison with HPLC or gas chromatography. HPTLC is often not only more effective in qualitative analysis, but can also be superior in quantitative analysis when utilized to the full.

THEORETICAL

The qualitative optimization of one analytical procedure by the help of another analytical method is not easy and often not free of systematic errors when the analytical basis of both procedures differs. Therefore, we propose the use of identical basic units for the comparison of qualitative data in thin-layer chromatography (TLC) and liquid chromatography (LC).

Although the following is probably well known to all chromatographers, we would like to emphasize two aspects of R_F values.

(1) R_F values are no measure of true chromatographic properties, but are mixed data, including non-chromatographic informations. The latter are so-called "dead" values. In normal dry or wet chamber developments, R_F values show additional specific "instrument- and procedure"-dependent influences. Therefore there is a basic difference between those R_F values and the data discussed in this paper, where even R_F data of 1.0 are measurable (by calculation of the front position according to a precise knowledge of applied phase volume). We call the latter R_F data "true".

(2) According to our experimental results with instrumental TLC, even in reversed-phase high-performance thin-layer chromatography (HPTLC) R_F values are prone to errors, unless measured under controlled gas phase conditions and controlled temperature conditions. Humidity is not the only problem inherent in measurement of true R_F data.

However, the results of circular developed TLC are comparable to those measured in identical chemical phase systems in columns, if k values are used for correlation, instead of R_F data. k is the ratio between the dead time and the adjusted retention time:

$$k = t_s/t_m$$

where t_s = retention time in the stationary phase, which is different for each compound that has different chromatographic properties; t_m = dead time, identical for each compound but dependent on non-chromatographic conditions. At first sight k data, being time-based parameters, seem to be unnecessarily complicated for expressing a result that can easily be measured in TLC. However, k data permit the simplest treatment for data comparison.

k data are related to time values and R_F values can also be expressed in terms of these time values. The analytical problem of a simple conversion of R_F into k is one of systematic errors of the basic time measurements. If, for instance t_m (which by definition is the time of movement of the liquid phase front) is changed by vapour phase condensation, the time relation is also in error. Loss of mobile phase by evaporation in a dry chamber tends to make t_m too large. Condensation of mobile phase in a saturated chamber makes t_m too small. In instrumental TLC, where the flow of mobile phase is quantitatively controlled and measured, systematic errors of time and time-relation measurements can be corrected. Thus, R_F into k value conversions no longer represent a serious problem for the analyst. Fig. 1 defines the basic units used.

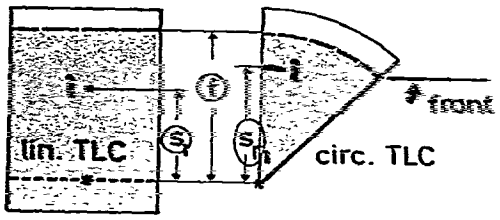
A column is compared with a thin layer, each of them containing an equal amount of stationary phase and wetted with an equal volume of mobile phase. Into both systems a substance i is introduced and chromatographed until the mobile phase front reaches the end of the column or a given line on the plate. Then we obtain the parameters given in Table I.

TABLE I

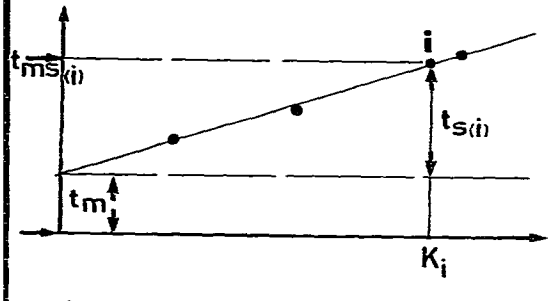
PARAMETERS IN COLUMN AND THIN-LAYER CHROMATOGRAPHY

Parameter	*Column*	*Layer*
Distance moved by mobile phase from the start (mm)	f_c	f_l
Distance substance i is transported from the start (mm)	s_c	s_l
"R_F value" of i	$R_{Fc} = s_c/f_c$	$R_{Fl} = s_l/f_l$

The "R_F values" of the column and the thin layer differ, owing to geometrical differences. The mobile phase–stationary phase relationship in both systems differs as the column is closed and the layer is "open" and because of the different forces required for phase movement.



$$R_{F_i} = s_i : f \quad = (s_{fi} : f)^2 \quad = t_m : t_{msi}$$



$$k_i = t_{s_i} : t_m = (t_{ms_i} - t_m) : t_m = K_i \cdot \text{const.}$$

Fig. 1. According to Geiss[1], R_F values of linear and circular TLC can be converted in each other only if the sample in circular TLC is applied precisely in the center. Time relations, R_F data and partition coefficients can be converted in each other only if chromatography is not disturbed by any gradient, including gradients produced by vapour phase condensation into the layer. K = partition coefficient.

If we continue the constant movement of the mobile phase in the column, *i.e.*, a constant flow-rate of mobile phase, until the substance i reaches the end of the column, it will be found that the relationship between the distances migrated is correlated with the time of migration, f_c now of course being greater than the length of the column. Therefore,

$$\frac{s_{c(i)}}{f_c} = R_{F(i)} = \frac{t_m}{t_{ms(i)}} \tag{1}$$

As $t_{ms(i)}$ = non-adjusted retention time = $t_{s(i)} + t_m$, it follows that

$$\frac{1}{R_{F(i)}} = \frac{t_{ms(i)}}{t_m} = \frac{t_{s(i)} + t_m}{t_m} = \frac{t_{s(i)}}{t_m} + \frac{t_m}{t_m} = k_{(i)} + 1$$

According to eqn. 1, it follows that

$$k_{i\,(\text{column})} = \frac{1}{R_{F\,(\text{column})}} - 1 \tag{2}$$

It is easy to obtain similar relationships when TLC data are measured under continu-

ous phase flow conditions (evaporation of the mobile phase at the plate end). It follows that

$$\frac{t_m}{R_{F(i)\ (TLC)}} = \frac{t_{ms(i)\ (TLC)}}{1}$$

and therefore

$$k_{i\ (layer)} = \frac{1}{R_{F\ (layer)}} - 1$$

For a given substance i, the R_F value on a given plate is independent of the migrated path and is a constant under isothermal isopolarity conditions (no gradient). This also applies to the results under column elution conditions, but

$$k_{i(column)} \neq k_{i(layer)} = \text{constant}$$

Therefore,

$$\frac{k_{i\ (layer)}}{k_{i\ (column)}} = \text{constant} = C$$

and

$$k_{i(layer)} = Ck_{i(column)} \tag{3}$$

The quantitative data transfer from plates to columns depends on how one measures the constant C.

With constant temperature conditions and chemically equivalent substances, the k relationships for two substances i and b will be identical in column and thin-layer chromatography, according to Fig. 2.

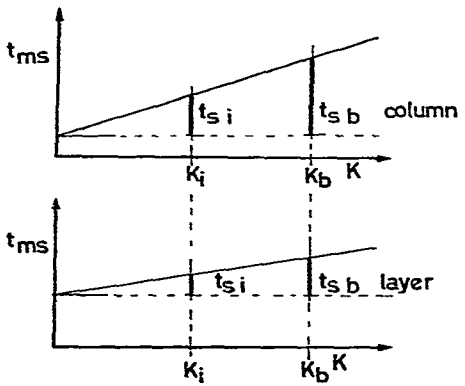


Fig. 2. Identical chromatographic substance conditions in mobile and stationary phase exist, when the partition coefficients, K, of at least two compounds are equal in column and layer chromatography. This does not include equal k values but equal k relations. t_{ms} = non-adjusted retention time; t_s = adjusted retention time.

Both chromatographic systems will have the same polarity, which can be expressed as a k relationship:

$$\frac{k_i}{k_b}\text{(column)} \equiv \frac{k_i}{k_b}\text{(layer)} \tag{4}$$

Comparing eqns. 4 and 3, we find the solution to the problem of transferring TLC data to LC systems:

$$k_{i(\text{layer})} = Ck_{i(\text{column})}$$

$$k_{i\ (\text{layer})} = \frac{k_{b\ (\text{layer})}}{k_{b\ (\text{column})}} \cdot k_{i\ (\text{column})} = C\,k_{i\ (\text{column})}$$

$$C = \frac{k_{b\ (\text{layer})}}{k_{b\ (\text{column})}}$$

Therefore

$$k_{i\ (\text{column})} = k_{i\ (\text{layer})} \cdot \frac{k_{b\ (\text{column})}}{k_{b\ (\text{layer})}} \tag{5}$$

Eqn. 5 expresses what should be done when TLC data are used as pilot data for optimization of LC:

(1) analyse under strictly chemically equal conditions on a thin layer and in a column a standard compound b and measure k for this standard compound in the column [$= k_{b(\text{column})}$] and on the thin layer [$= k_{b(\text{layer})}$];

(2) measure the k values of the substances of interest on the plate;

(3) calculate

$$C = \frac{k_{b\ (\text{layer})}}{k_{b\ (\text{column})}}$$

(4) multiply any k_i value from the TLC analysis by C and calculate the k values valid for the column;

(5) as $k_i = t_{s(i)}/t_m$ and $t_{s(i)} + t_m$ = total elution time for compound i, it can easily be calculated which peak represents which substances because the non-adjusted retention time can be pre-calculated:

$$t_{ms(i)} = t_{s(i)} + t_m = k_i \cdot t_m + t_m$$

$$t_{ms(i)} = t_m(k_i + 1)$$

This can easily be measured in column chromatography; $k_{i(\text{column})}$ is calculated according to eqn. 5.

Example

$t_{m(\text{column})} = 25$ sec

$k_{i(\text{layer})} = 7.75$; $7.75 + 1 = k_i + 1$

$\frac{k_{b\ (\text{column})}}{k_{b\ (\text{layer})}}$ (*e.g.*, for caffeine as substance b) $= 0.70$

Elution time for the substance i with $k_{i(\text{column})} = 7.75 \cdot 0.70 = 5.4$ is $(5.4 + 1) \cdot 25$ sec = 160 sec.

Of course, we can now re-calculate R_F data on the plate: $k_i = 7.75$ corresponds with $R_F = 1/(7.75 + 1) = 0.114$.

Note: R_F data measured by circular HPTLC have to be corrected into linear R_F data according to Geiss[1]: $[R_{F(\text{circular})}]^2 = R_{F(\text{linear})}$.

DISCUSSION

Although the theoretical background is very simple when only one set of real chromatography-dependent data is considered, *i.e.*, k instead of the R_F value, in practice it is often a problem to guarantee completely equivalent chemical conditions for the stationary phase in columns and thin-layer plates. And this is an essential condition. Binders in the thin layer or metal walls in the column are only one source of inequivalence.

Only under true isocratic conditions can R_F data from TLC be recalculated into k units. If inappropriate conditions results in incorrect R_F values owing to evaporation, condensation of the mobile phase or pre-saturation of the stationary phase with the mobile phase, the k data are systematically incorrect. With evaporation the calculated k data are too small, while with pre-saturation they are too large.

It was found by experiment that accurate R_F values which correlate with the k data can be measured only under critical phase control conditions. Further, it is obvious that optimization of TLC is of value only when conditions can be found which reduce the analysis time, which means larger k values have to be pre-calculated accurately.

A value of $k = 10$ from column analysis corresponds to an R_F value of approximately 0.1 on a thin-layer plate with equal polarity and temperature. This is a level which experimentally is attained only by the circular TLC technique. It is therefore of great help to have instrumental circular HPTLC available, as this technique guarantees accurate k data only in the higher region of interest in HPLC optimization, *i.e.*, at R_F values lower than 0.1 (see ref. 2).

Fig. 3 shows the use of this procedure, comparing R_F k data on a C_{18} reversed-phase high-performance plate with column k data on the same stationary phase. The conditions are given in the figure caption.

ENVIRONMENTAL ANALYSIS ON REVERSED-PHASE HIGH-PERFORMANCE PLATES

Environmental analysis requires enrichment and a high separation power together with highly specific detection at a sufficient sensitivity. The highest separation power is given when two- or multi-dimensional separation is used. Only two-dimensional TLC techniques offer high separation power at the cost of only twice the analysis time, whereas two-dimensional column separations mean multiple time consumption, as shown in Fig. 4.

Environmental analyses often require sample preparation, clean-up and enrichment. All of these procedures can be carried out on-plate and HPTLC is therefore most effective in environmental analysis. According to the stability and consistency of chemically bonded phases, enrichment is no problem on thin-layer plates. Otherwise,

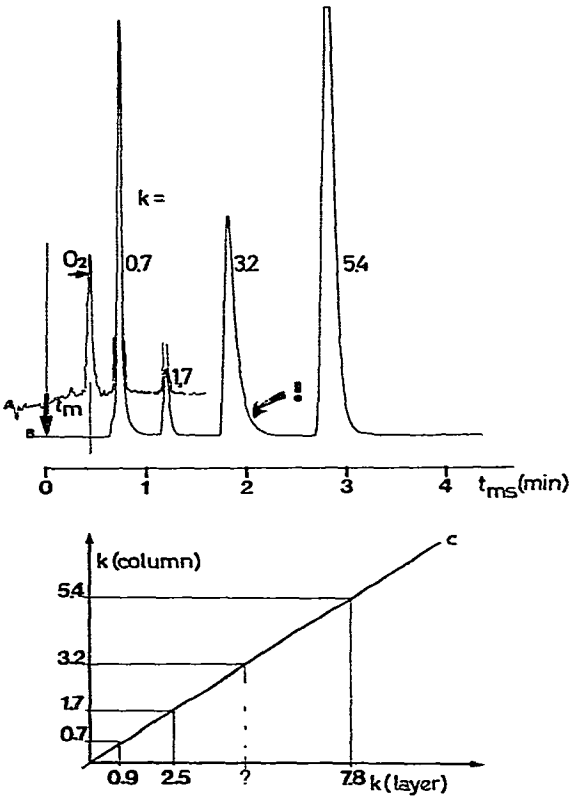


Fig. 3. If equal chemical conditions in layer and column chromatography exist, k column data correlate linearly with k layer data, as shown in this figure. Mobile phase: acetonitrile, 2 ml/min, in HPLC and HPTLC; stationary phase: LiChrosorb RP-8; 5 μm, No. 761057 Merck (Darmstadt, G.F.R.) ready-made column in an Institute of Chromatography (Bad Dürkheim, G.F.R.) separation cassette; RP-8 ready-made high-performance plate (Dr. H. Halpaap, Merck; experimental plates); sample: solution of a headache pill. Detection: UV, 254 nm.

enrichment would drastically change the adsorption behaviour of adsorbing TLC plates at the enrichment stage owing to the presence of large amounts of mostly polar compounds, such as water and salts. Reversed-phase materials in addition often have higher sample capacities. Fig. 5 describes a simple enrichment procedure carried out under nitrogen and in the absence of UV light, which is of importance in practical environmental analysis. Fig. 6 shows how the substances of interest can be separated in step 1 from "waste", and Fig. 7 shows how the optimization of the final separation is effected by simultaneous multi-phase development[3].

Having found the optimal separation conditions for the substances of interest, subsequent two-dimensional separation is carried out in a simple, flat N-chamber which takes care of the need to measure R_F values accurately (no pre-separation falsifies the R_F values) and which permits the analysis of 60 samples in 4 min together on one plate under optimized conditions.

The speed of quantitation is a bottleneck. As HPLTC quantitation is superior to HPLC quantitation in almost all instances as a result of the virtually unlimited

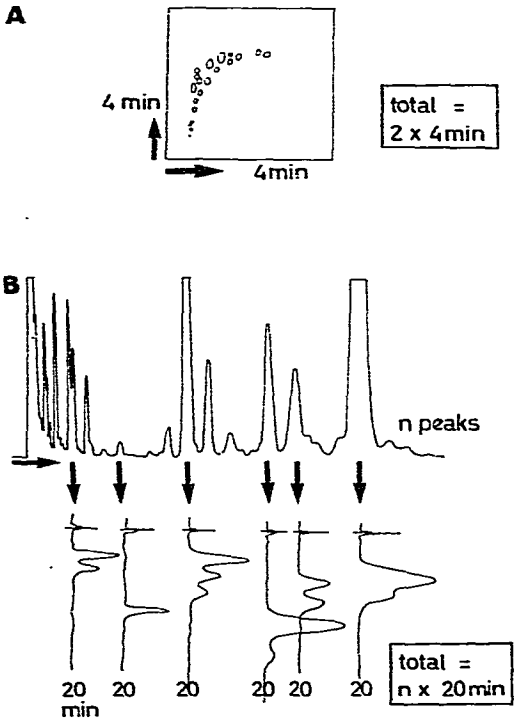


Fig. 4. Comparison of two-dimensional TLC (A) and elution chromatography (B). Separation number (number of fully separated peaks in the range of $k = 0$ to $k = 10$) is identical in TLC and LC. Two-dimensional total separation number in both systems: $20 \times 20 = 400$ peaks; analysis time in TLC: $2 \times 4 = 8$ min; analysis time in LC: 20×20 min $= 400$ min, for a comparable number of data.

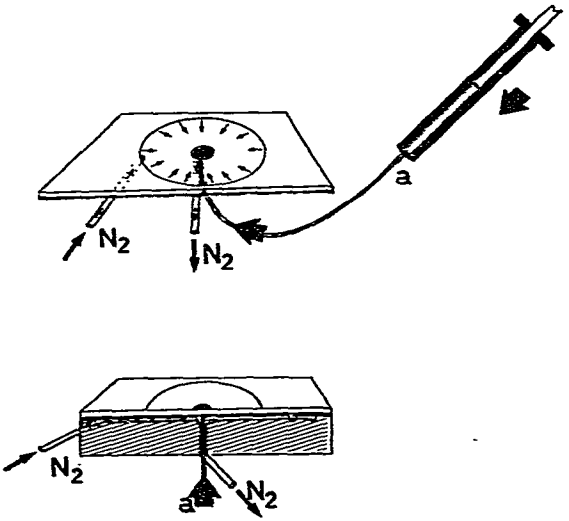


Fig. 5. Instrumental circular TLC: solvent (mobile phase) is fed by a syringe (a) onto the plate center; nitrogen flows counter current. This technique is useful for maximum volume enrichment of non-volatile traces in the plate center. Sample and clean-up solvent for the pre-separation of substances of interest from dirt flow from a.

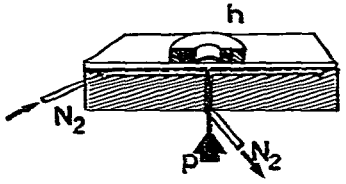


Fig. 6. Enrichment and focusing of traces from maximum volume solutions in a ring similar to the ring oven technique by Weisz[6] with a solvent (p), having high elution power, but high vapour pressure as well. h = ring heated.

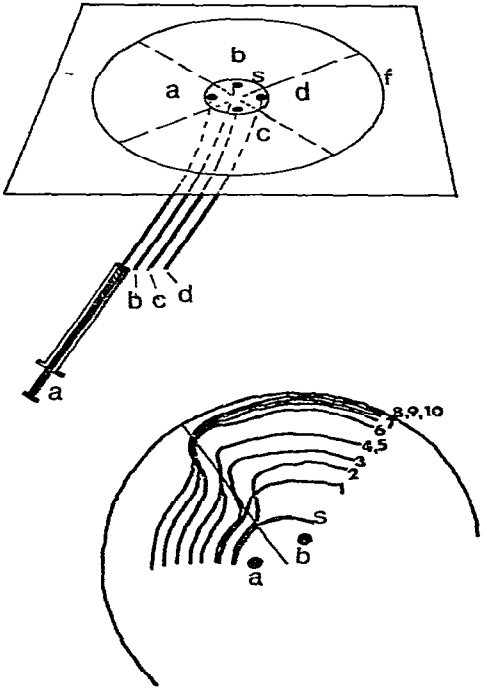


Fig. 7. Multiphase simultaneous HPTLC separation of a sample, focused as a circle around three, four or more capillary inlets for mobile phases. Lower part of the figure: transition area in the range of phase a to b. The angle is a measure of the relative phase composition; 50:50 % (v/v) is on the thin line. Too different phases, *e.g.* methanol and pentane, cannot be used at the same plate, but mixtures sufficiently similar in physical and chemical behaviour can be used simultaneously. Strong differences in viscosity disturb a clear transition area.

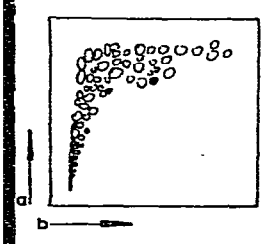


Fig. 8. Two-dimensional 5 × 5 cm HPTLC separation of enriched air particulate extracts (collected in Birmingham, Ala., U.S.A.; by courtesy of EPA, U.S.A.). ●, Benzo[*a*]pyrene. Mobile phases: a, methanol–water (97:3); b, methanol–acetonitrile (9:1). Stationary phase: C_{18} chemically bonded. Sample applied: 200 nl from a 1000-l air sample enriched in benzene. Analysis time: 2 × 4 min. The spots were fluorescent and were detected by the eye.

possibilities of specific detection, the time factor is restrictive. Modern electronics and integrators are too slow for the full use of this powerful analytical procedure. Fig. 8 shows an example of the analysis of an air particulate extract from air dust particles collected in Birmingham, Ala., U.S.A. This analysis was very difficult to carry out by gas chromatography, even when using capillaries and multi-detection devices including a mass spectrometer[4]. Procedures for collecting and treating such samples prior to analysis are available[5].

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