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COMPARISON OF REVERSED-PHASE THIN-LAYER AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF FLAVONOID COMPOUNDS

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SUMMARY

The reversed-phase high-performance liquid (HPLC) and thin-layer chromatographic (TLC) behaviour of flavonoids with methanol, tetrahydrofuran and acetonitrile as organic modifiers was compared. Twenty-six different correlation cases, together with their statistical parameters, are presented and discussed. Both TLC and HPTLC plates were considered. The dependence of TLC incremental R_m values from different group substitutions on solvent type, solvent composition and type of plate is considered. A method for establishing HPLC gradient elution conditions by using TLC data is discussed.

INTRODUCTION

Reversed-phase high-performance liquid (HPLC) and thin-layer chromatography (TLC) are well established separation methods in flavonoid analysis[1–12]. In previous papers the behaviour of flavonoid compounds in several reversed-phase HPLC partition systems differing in (1) column type, (2) acid modifier, (3) organic modifier [methanol, tetrahydrofuran (THF) and acetonitrile] was considered and the selectivity properties with reference to isocratic and gradient elution separations were discussed[13–16].

Despite the many publications on TLC flavonoid analysis[6–12], no general and systematic study of the influence of experimental variables such as the solvent, composition and type of plate could be found. In this work these aspects were examined with reference to reversed-phase systems and the relationship between HPLC and TLC was studied. The last approach, because of its many useful practical implica-

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tions[17–23], appears to be promising for solving many complex problems of flavonoid analysis.

EXPERIMENTAL

The TLC measurements were carried out on three different reversed-phase C_{18} pre-coated layers: (a) TLC KC18F (Cat. No. 4803-800) (Whatman, Clifton, NJ, U.S.A.), (b) TLC pre-coated plate RP-18F_{254}S (Cat. No. 15423) (Merck, Darmstadt, F.R.G.) and (c) HPTLC pre-coated plate RP-18WF_{254}S (Cat. No. 13124) (Merck); these are referred to as TLC (W), TLC (M) and HPTLC (M), respectively.

The following parameters were kept constant: size of the plates (10 cm × 10 cm), solvent volume (20 ml) in the developing tank (10 cm high × 20 cm × 5 cm), distance of the starting line from the bottom (1 cm) and distance of development (5 cm). After the application of the spots (0.1–0.2 μl) of standard solutions, ascending development was carried out at room temperature. The spots were located under UV light by quenching of the fluorescence at 254 nm. R_F measurements were repeated four times on the same plate washed with methanol after each development. No drift in the retention data was observed and the standard error over the mean R_m value was always between 0.07 and 0.03.

The solvents were of HPLC grade from Rudi-Pont (Parsippany, NJ, U.S.A.). Methanol, acetonitrile and THF were utilized as organic modifiers in binary mixtures with water. The aqueous phase was buffered at pH 2–3 in 80 m*M* citric or acetic acid – 8 m*M* disodium hydrogenphosphate (Carlo Erba, Milan, Italy). The selected flavonoid standards were Extrasyntese (Genay, France), used as received and dissolved in ethanol (HPLC grade) to give 1000 ppm solutions. The selected standards represent flavones, flavonols, flavanones and glycosides and are listed in Table I.

TABLE I

LIST OF FLAVONOID COMPOUNDS STUDIED

No.	*Compound*	*No.*	*Compound*
1	Acacetin	10	Luteolin 7-O-glucoside
2	Apigenin	11	Morin
3	Apigenin 7-O-glucoside	12	Naringenin
4	Apiin	13	Quercetagetin
5	Chrysin	14	Quercetin
6	Chrysoeriol	15	Quercitrin
7	Eriodictyol	16	Rutin
8	Galangin	17	Myricetin
9	Luteolin	18	Vitexin

All the HPLC data were obtained on a μBondapak C_{18} column (Waters, Milford, MA, U.S.A.) (W1 and W3 data from refs. 15 and 16). Retention data with a given organic modifier were obtained on the same column. HPLC data for vitexin and myricetin were obtained on column W3 by following the experimental procedure in refs. 15 and 16.

THEORETICAL

The comparison between TLC and HPLC is based on the study of the relationship

$$\log k' = f(R_M) \tag{1}$$

where k' is the capacity factor and R_M is defined as

$$R_M = \log(1/R_F - 1) \tag{2}$$

The most commonly used form of eqn. 1 is the linear relationship

$$\log k' = A + BR_M \tag{3}$$

and using different test compounds it has proved to be followed by different HPLC and TLC systems[22]. When $A = 0$ and $B = 1$ both the partition processes are the same and

$$\log k' = R_M \tag{4}$$

Any departure from this ideal behaviour may have a simple thermodynamic explanation. In fact, remembering that

$$k' = KV_s/V_m \tag{5}$$

where V_s and V_m are the stationary phase and mobile phase volumes, respectively, and K is the distribution constant, an A value other than zero can mean either a different phase ratio value or a multiplying factor acting on K. As

$$\Delta\mu^0 = -RT \ln K \tag{6}$$

where $\Delta\mu^0$ is the standard Gibbs free energy change for a solute in the mobile phase passing into the stationary phase, a multiplying factor on K means a constant shift $\Delta(\Delta\mu^0)$ for all the solute samples. A non-unit value of B in eqn. 3 appears as a constant multiplying factor over the whole scale of the free energy of transfer, implying more complex differences in the thermodynamic partition properties of the two systems being compared. In order to understand better the underlying changes in solute–stationary phase interactions, the comparison of TLC and HPLC can be further analysed by studying the following relationship:

$$\Delta \log k' = f(\Delta R_M) \tag{7}$$

where $\Delta \log k'$ and ΔR_M are calculated over solute pairs differing in some characteristic substituent groups. Such a study can highlight specific polarity, lipophilicity, hydrogen bonding and dipolar interaction changes of the two chromatographic systems being compared.

In addition to these purely thermodynamic effects, other phenomena pertaining to the chromatographic process itself may be responsible for the departure from the theoretical relationship with unit slope and zero intercept, which is observed even when the same material is used for the HPLC column packing and the TLC layer. In fact, it is well known that there is no perfect equivalence between TLC development and HPLC elution and many physical peculiarities have been described and discussed, *e.g.*, the roles of the interphases, capillary forces and demixing processes[24,25]. As these effects are dependent on the mobile phase volume composition (Φ), some insight into them can be attained by studying the log k'–R_M and $\Delta\log k'$–ΔR_M relationships with changing Φ. For example, if $\Delta\log k'$ group contributions in an HPLC system are independent of Φ and the TLC and HPLC systems are identical, the ΔR_M values will be not affected by effects due to changes in the mobile phase composition and their values will be equal to $\Delta\log k'$. Obviously many other cases could be described implying a complex integral effect of R_M with changing Φ. For the above arguments, an exhaustive description of the TLC *vs.* HPLC relationship must take into account a broad range of different independent variables if the aim is to achieve precision and accuracy and also to check different theoretical hypotheses.

RESULTS AND DISCUSSION

All the HPLC reference data taken into consideration in this work were obtained on a μBondapak C_{18} column. In a previous study[15] it was shown that no substantial difference in the relative retentions of flavonoid compounds is observed when using C_8 or C_{18} bonded phases from different manufacturers, so the results of this study can be easily extended to these different systems. The only parameter affected will be A in eqn. 3, which reflects the phase volume ratio.

In Table II the results of 26 linear HPLC–TLC correlations are reported. In this study six different factors were considered: (a) different TLC layers (TLC, HPTLC); (b) different manufacturers (W,M); (c) different solvent volume fraction (Φ); (d) different solvents (methanol, acetonitrile, THF); (e) different acid modifier with the same solvent (citric and acetic acid in methanol); and (f) repeatibility (systems 16, 17 and 18, 19 in Table II).

A number of compounds generally between ten and twenty were employed in establishing HPLC–TLC correlations. R_M values in the range -0.2 to 1.2 were considered. This range is different from that recommended for physico-chemical studies (-0.6 to 0.6), but it is commonly accepted for correlation studies[26]. In addition, both the log k' and R_M ranges were homogeneously covered. As a general remark, it was observed that M layers exhibit lower retentions than W layers. Good correlation coefficients (greater than 0.95) were observed. Minor exceptions were observed at water concentrations higher than 50% in the eluent phase and with methanol as organic modifier both on TLC and HPTLC plates (systems 1,2 and 15 in Table II). These last cases are probably due to demixing phenomena in the mobile phase, which was indeed observed experimentally.

Disregarding the above-mentioned demixing effects, systematic differences are observed between TLC and HPTLC plates in methanol. For the first system a unit slope with a low and nearly constant intercept (-0.20) is observed in most instances whether on plates from different manufacturers or with different acid modifiers in the

TABLE II

HPLC–TLC CORRELATION RESULTS ACCORDING TO EQUATION LOG $k' = A + BR_M$
Acid modifier: acetic acid except where specified otherwise.

System No.	*Plate*	*Source*	*Solvent*	*Φ (%)*	*A*	*B*	*R**	$\sigma_{y.x}$**
1	TLC	W	Methanol	40	−0.20±0.21	1.03±0.24	0.89	0.12
2	TLC	W	Methanol	45	−0.16±0.22	0.80±0.28	0.76	0.14
3	TLC	W	Methanol	50	−0.19±0.04	0.97±0.06	0.98	0.06
4	TLC	W	Methanol***	50	−0.43±0.05	1.00±0.08	0.97	0.09
5	TLC	W	Methanol	55	−0.19±0.03	0.99±0.06	0.99	0.05
6	TLC	W	Methanol	60	−0.13±0.02	0.92±0.05	0.99	0.07
7	TLC	W	Methanol***	60	−0.23±0.02	0.96±0.05	0.99	0.05
8	TLC	M	Methanol	50	−0.22±0.02	1.03±0.03	0.99	0.02
9	TLC	M	Methanol	55	−0.24±0.03	1.02±0.07	0.98	0.05
10	TLC	M	Methanol	60	−0.42±0.02	1.16±0.05	0.99	0.06
11	TLC	W	THF	45	−0.36±0.03	1.56±0.07	0.99	0.07
12	TLC	W	Acetonitrile	40	−0.04±0.02	1.06±0.04	0.99	0.06
13	TLC	M	THF	45	−0.41±0.01	1.55±0.02	0.99	0.02
14	TLC	M	Acetonitrile	40	−0.14±0.01	1.03±0.03	0.99	0.04
15	HPTLC	M	Methanol	45	−0.16±0.30	0.85±0.41	0.64	0.16
16	HPTLC	M	Methanol	50	−0.27±0.07	1.19±0.10	0.97	0.08
17	HPTLC	M	Methanol	50	−0.31±0.05	1.17±0.08	0.98	0.07
18	HPTLC	M	Methanol	55	−0.27±0.04	1.36±0.07	0.98	0.08
19	HPTLC	M	Methanol	55	−0.21±0.04	1.37±0.08	0.98	0.09
20	HPTLC	M	Methanol	60	−0.38±0.03	1.43±0.11	0.97	0.09
21	HPTLC	M	THF	40	−0.61±0.04	1.05±0.04	0.99	0.05
22	HPTLC	M	THF	45	−0.66±0.03	1.19±0.04	0.99	0.05
23	HPTLC	M	THF	50	−0.35±0.02	0.98±0.05	0.98	0.05
24	HPTLC	M	Acetonitrile	40	−0.39±0.03	1.49±0.05	0.99	0.03
25	HPTLC	M	Acetonitrile	45	−0.25±0.05	1.52±0.09	0.98	0.06
26	HPTLC	M	Acetonitrile	50	−0.14±0.02	1.46±0.98	0.99	0.05

* R = Correlation coefficient.
** $\sigma_{y.x}$ = Standard error of regression.
*** Citric acid as acid modifier.

mobile phase. The only exception is the lower intercept (−0.43) with citric acid modifier at 50% methanol (system 4 in Table II). This last finding, if related to the abrupt change in the HPLC solvent strength observed at this particular organic modifier composition[13,14], may not be particularly remarkable.

In the HPTLC system, slopes always greater than unity with a significantly lower intercept are observed (systems 16 and 17 in Table II). The former analysis suggests that in contrast to HPLC, TLC plates in methanol behave as a nearly ideal system, whereas departures from ideality are apparently exhibited by HPTLC plates. If, on the other hand, different organic modifiers are also considered, one can see that this sharp differentiation does not reappear. In fact, with 40% acetonitrile a unit slope is observed on TLC plates (systems 12 and 14) but with 45% THF (systems 11 and 13) on the same type of plates the slope is 1.6. The reverse is observed for HPTLC plates, but with the THF intercept being very negative (systems 24–26 for acetonitrile and 21–23 for THF). Hence one can conclude that the nearly ideal behaviour (unit slope and low intercept value) observed with TLC plates is the combined result of the use of

TABLE III

SUBSTITUENT GROUP CONTRIBUTIONS (ΔR_M OR Δ LOG k') TO RETENTION WITH DIFFERENT C_{18} REVERSED-PHASE CHROMATOGRAPHIC SYSTEMS

Methanol as organic modifier with different acid modifiers. Data reported as mean ΔR_M or Δ log k' values with their standard errors. Three or four different mobile phase compositions in the reported composition range were considered.

Group contribution	*Compounds*	*TLC (W) (citric acid), 0.40–0.60**	*TLC (W) (acetic acid), 0.40–0.60**	*TLC (M) (acetic acid), 0.45–0.65**	*HPTLC (M) (acetic acid), 0.45–0.65**	*HPLC*** *(citric acid), 0.40–0.70**	*HPLC**** *(acetic acid), 0.40–0.70**
3-OH	14–9	−0.18±0.07	−0.12±0.03	−0.14±0.04	−0.08±0.02	−0.15±0.05	−0.13±0.06
6-OH	13–14	−0.87±0.07	−0.75±0.13	–	–	−0.94±0.09	−0.80±0.11
3′-OH	9–2	−0.25±0.04	−0.29±0.03	−0.25±0.03	−0.19±0.06	−0.20±0.04	−0.22±0.04
3′-OH	10–3	−0.17±0.05	–	−0.13±0.03	−0.11±0.04	−0.21±0.14	−0.18±0.01
3′-OH	7–12	−0.26±0.02	−0.26±0.02	−0.25±0.04	−0.14±0.04	−0.24±0.02	−0.24±0.03
4′-OH	2–5	−0.40±0.09	−0.37±0.08	−0.31±0.03	−0.14±0.01	−0.30±0.08	−0.29±0.03
5′-OH	17–14	−0.30±0.04	−0.30±0.05	–	–	–	−0.27±0.01
3′-OCH_3(-HO)	6–2	0.06±0.02	0.03±0.03	0.03±0.02	0.04±0.02	0.06±0.01	0.06±0.01
4′-OCH_3	1–5	0.14±0.03	0.11±0.06	0.16±0.03	0.16±0.03	0.13±0.03	0.14±0.01
2,3-Unsaturation	12–2	−0.42±0.03	−0.43±0.05	−0.43±0.07	−0.34±0.06	−0.47±0.13	−0.39±0.02
2,3-Unsaturation	7–9	−0.48±0.06	−0.41±0.06	−0.45±0.09	−0.38±0.03	−0.51±0.12	−0.33±0.11
3-Glycoside (rhamnose)	15–14	−0.30±0.02	−0.33±0.03	−0.28±0.04	−0.18±0.02	−0.22±0.07	−0.35±0.07
3-Glycoside (rutinose)	16–14	−0.52±0.04	−0.52±0.04	−0.48±0.04	−0.31±0.04	−0.47±0.03	−0.42±0.04
7-Glycoside (glucose)	3–2	−0.73+0.01	−0.72±0.06	−0.69±0.07	−0.49±0.07	−0.77±0.18	−0.68±0.03
7-Glycoside (glucose)	10–9	−0.70±0.09	–	−0.60±0.10	−0.41±0.05	−0.71±0.04	−0.71±0.04
7-Glycoside (apiosylglucose)	4–2	−0.85±0.07	−0.77±0.04	−0.75±0.06	−0.53±0.06	−0.77±0.06	−0.77±0.03
8-C-Glycoside (glucose)	18–1	−1.14±0.06	−1.09±0.08	–	–	–	−1.06±0.04

* Mobile phase composition range.

** All the reported data except for 5′-OH and 8-C-glycoside are taken from ref. 15.

TLC plates and methanol as organic modifier in the Φ range 50–60%, as no other plate–solvent combination gives this effect. In Fig. 1a and b the above-described main types of behaviour are shown; Fig. 1a is an example of an "ideal' correlation with TLC–methanol systems and Fig. 1b is an example of the correlation on HPTLC plates with a slope significantly greater than unity.

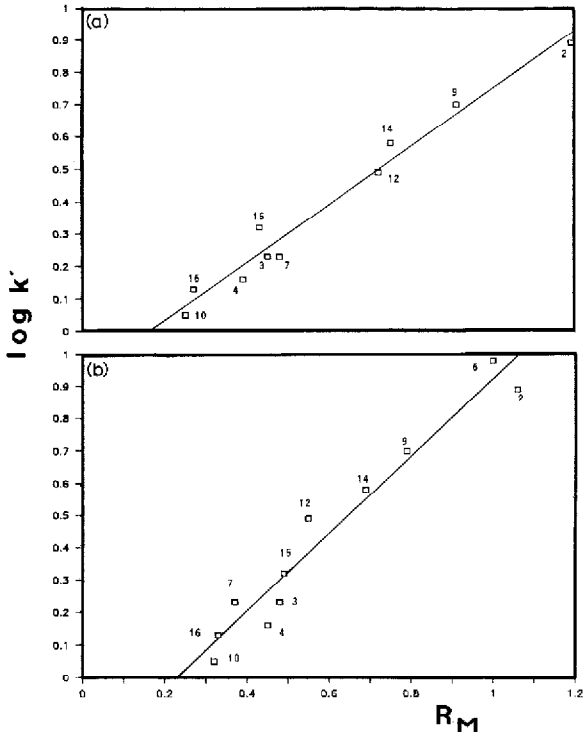

Fig. 1. HPLC–TLC correlation; 50% methanol as organic modifier and acetic acid as acid modifier. The numbers refer to the compounds listed in Table I. (a) TLC (W); (b) HPTLC (M). HPLC data from ref. 15.

The correlation between the $\Delta\log k'$ and ΔR_M values responsible for a particular substituent group within the molecule can help to explain the observed behaviour (see Table III). The group contributions in the methanol systems, in HPLC found to be independent of the volume fraction Φ, column type and acid modifier (citric or acetic)[15], are analysed first (Table III). One can see that the agreement between $\Delta\log k'$ and ΔR_M values is very satisfactory for TLC plates and only small systematic differences are observed with HPTLC. In addition, the insensitivity towards Φ is exhibited by all the TLC systems, as revealed by the low standard errors reported in Table III.

Fig. 2a shows the strict correlation between HPLC $\Delta\log k'$ and TLC ΔR_M data, with a slope equal to unity and an intercept nearly equal to zero. Fig. 2b shows the correlation with HPTLC data. In the latter instance the intercept is again nearly equal to zero but the slope is greater than unity. These last findings can give some insight

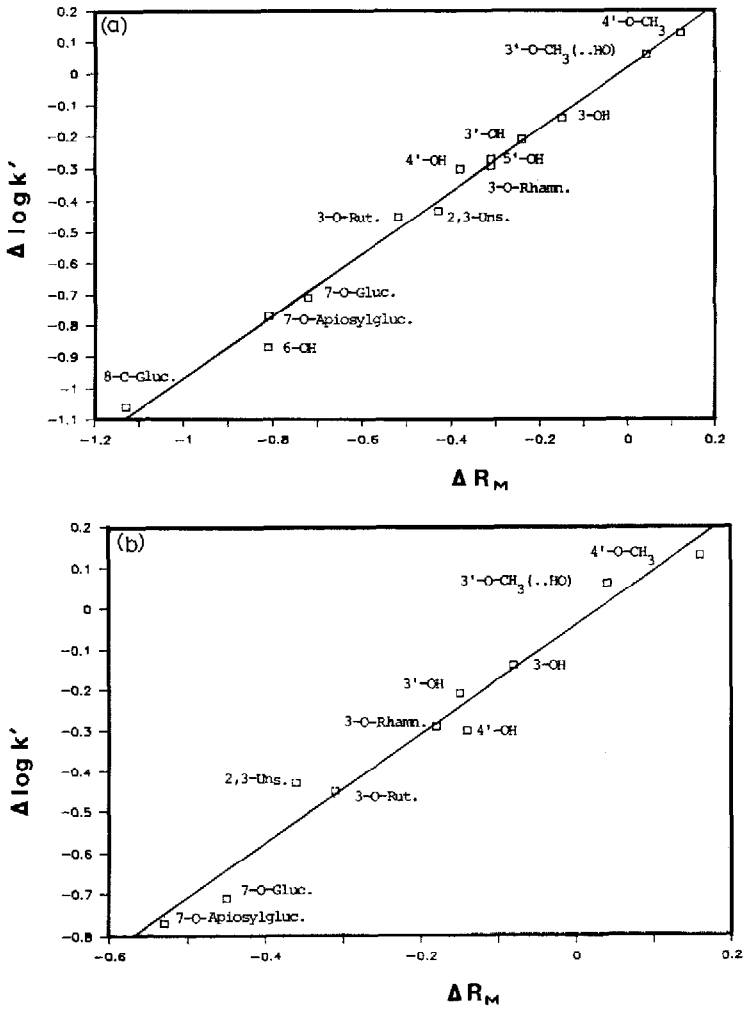

Fig. 2. ΔLog k' (HPLC) *vs.* ΔR_M (TLC) for (a) TLC (W) and (b) HPTLC (M). See Table III. Abbreviations: Gluc. = glucose; Rhamn. = rhamnose; Rut. = rutinose; Uns. = unsaturation.

into the different, previously mentioned behaviour of TLC and HPTLC plates. The constancy of the group retention contributions in the first instance means an equivalence of the partition properties of TLC and HPLC systems. The HPTLC plates appear instead to be about 1.5 times more polar than the two previous systems. At this point one might ask whether this polarity increase is a pure thermodynamic effect or rather an apparent effect due to the composition changes during the chromatographic development. As mentioned in the Theoretical section, we believe that methanol as organic modifier is probably unable to cause such an effect because in it the group contributions are largely insensitive towards composition with all the partition systems examined (HPLC, TLC, HPTLC). Hence it can be assumed that there is an effective polarity difference between TLC and HPTLC plates without ruling out

TABLE IV

SUBSTITUENT GROUP CONTRIBUTIONS [ΔR_M (TLC) OR ΔLOG k' (HPLC) WITH 45% TETRAHYDROFURAN IN THE MOBILE PHASE
Acetic acid as acid modifier. HPLC data taken from ref. 16.

Group contribution	*Compounds*	*TLC (W)*	*TLC (M)*	*HPTLC (M)*	*HPLC*
3-OH	8–5	0.15	0.12	0.13	0.20
3-OH	14–9	0.08	0.09	0.10	0.12
6-OH	13–14	−0.47	−0.48	–	−0.70
3′-OH	9–2	−0.05	−0.04	−0.12	−0.07
3′-OH	10–3	−0.04	−0.04	−0.06	−0.04
3′-OH	7–12	−0.06	−0.06	–	−0.11
4′-OH	2–5	−0.11	−0.14	−0.11	−0.20
3′-OCH_3(-HO)	6–2	−0.03	−0.04	−0.08	−0.05
4′-OCH_3	1–5	−0.01	−0.03	−0.06	−0.02
2,3-Unsaturation	12–2	0.07	0.05	–	0.13
2,3-Unsaturation	7–9	0.06	0.03	0.04	0.09
3-Glycoside (rhamnose)	15–14	−0.28	−0.37	−0.43	−0.55
3-Glycoside (rutinose)	16–14	−0.54	−0.57	−0.70	−0.72
7-Glycoside (glucose)	3–2	−0.35	−0.38	−0.54	−0.62
7-Glycoside (glucose)	10–9	−0.34	−0.38	−0.48	−0.59
7-Glycoside (apiosylglucose)	4–2	−0.40	−0.45	−0.64	−0.72

gradient composition effects during development. A full explanation of such a hypothesis would require a systematic investigation by means of an extended polarity scale of both the eluent and test sample compounds. This aspect is outside the scope of this paper.

In Tables IV and V the dependence of the group contributions on the partition

TABLE V

SUBSTITUENT GROUP CONTRIBUTIONS [ΔR_M (TLC) OR ΔLOG k' (HPLC) WITH 40% ACETONITRILE IN THE MOBILE PHASE
Acetic acid as acid modifier. HPLC data taken from ref. 16.

Group contribution	*Compounds*	*TLC (W)*	*TLC (M)*	*HPTLC (M)*	*HPLC*
3-OH	8–5	0.00	0.11	0.05	0.08
3-OH	14–9	0.02	0.04	0.13	0.03
6-OH	13–14	−0.37	−0.38	–	−0.37
3′-OH	9–2	−0.20	−0.21	−0.15	−0.22
3′-OH	10–3	−0.04	−0.04	−0.05	−0.01
3′-OH	7–12	−0.16	−0.19	–	−0.22
4′-OH	2–5	−0.42	−0.43	−0.29	−0.45
3′-OCH_3(-HO)	6–2	0.00	0.02	0.02	0.03
4′-OCH_3	1–5	0.00	0.04	0.03	0.05
2,3-Unsaturation	12–2	−0.08	−0.05	–	0.01
2,3-Unsaturation	7–9	−0.04	−0.03	−0.05	0.01
3-Glycoside (rhamnose)	15–14	−0.28	−0.29	−0.23	−0.38
3-Glycoside (rutinose)	16–14	−0.38	−0.39	−0.39	−0.38
7-Glycoside (glucose)	3–2	−0.48	−0.53	−0.41	−0.58
7-Glycoside (glucose)	10–9	−0.32	−0.36	−0.31	−0.37
7-Glycoside (apiosylglucose)	4–2	−0.52	−0.57	−0.41	−0.56

system with THF and acetonitrile, respectively, are presented. If these data are compared with those for methanol (Table III), it can be seen that there is general agreement between the HPLC and TLC group contribution patterns on changing organic modifier. Hence the solvent selectivity properties and their usefulness in flavonoid identification, previously considered for HPLC[16], are conserved in TLC systems. If these data are analysed more closely, it can be seen that no distinct behaviour is followed by a particular type of plate or a particular organic modifier, the only major finding being the constant behaviour of both types of TLC (W, M) plates. In addition, the differences between ΔR_M and $\Delta \log k'$ data are always more detectable than those found in methanol. This finding, common to all types of plates, is probably due to the marked dependence of the group contributions on Φ with these solvents, resulting from the effects of composition changes on the plate, although polarity differences of the HPTLC layer cannot be ruled out. In support of this hypothesis, one can see the dependence of ΔR_M and $\Delta \log k'$ on Φ, compared in Fig. 3 for the HPTLC plate. For certain group contributions a parallel behaviour is observed but for others (see, *e.g.*, 3-O-Rut.) more complex behaviours with intercrossing occur, which admittedly may result from both polarity and composition gradient effects.

Let us now consider the practical relevance of the useful effects. A slope greater than unity means substantial compression of the useful HPLC chromatographic space (*i.e.*, $1 < k' < 10$) when it is projected over the corresponding TLC or HPTLC space. Hence a slope of unity would be preferable because this adverse effect would be avoided. If in addition the intercept is near to zero, retention data can be easily transferred from HPLC to TLC and *vice versa*. Another interesting property of TLC plates is their use in compound identification: as the ΔR_M values are equal to $\Delta \log k'$ to within ± 0.03 unit in the $\Delta \log k'$ range 0–0.40, relative retention in HPLC can be

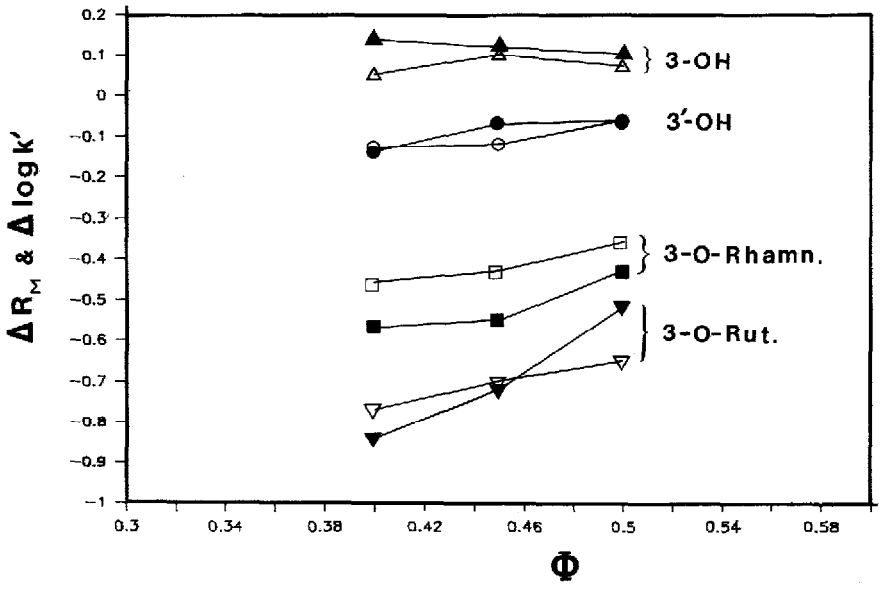

Fig. 3. ΔR_M (TLC) and $\Delta \log k'$ (HPLC) dependence with THF. TLC data were obtained on HPTLC plates. HPLC data were obtained from ref. 16. Closed symbols, HPTLC; open symbols, HPLC.

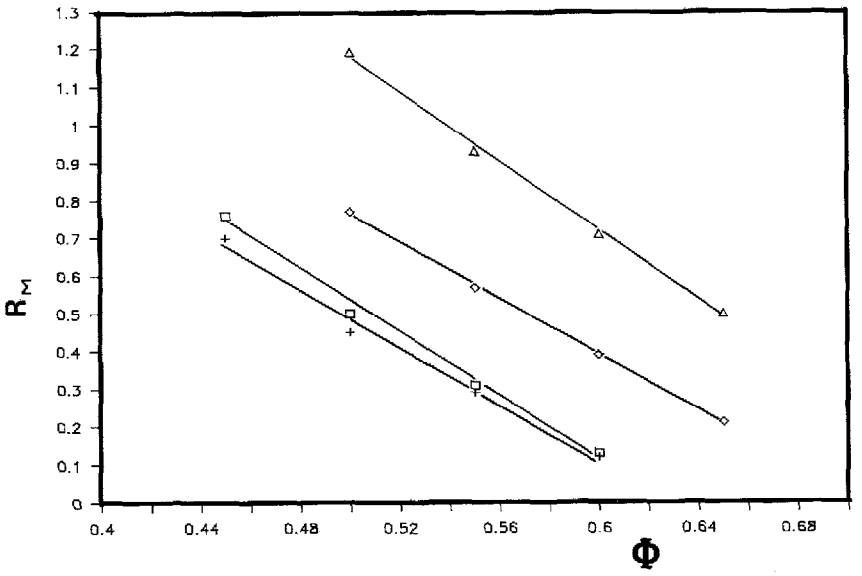

Fig. 4. R_M *vs.* methanol concentration (Φ) in the mobile phase. TLC (M). Compounds: △ = apigenin; ◇ = quercetin; □ = quercitrin; + = eriodictyol.

predicted with an accuracy of ±5%. On both TLC and HPTLC plates retention requires a calibration graph and, in the most favourable instance with log k' = 0.5 (k' = 3), the standard error of the fitting (Table II) implies an accuracy of 12% in the absence of demixing effects.

Another useful application of the TLC system with methanol would be its usefulness in establishing HPLC gradient elution conditions. It is well known that this procedure consists in determining the useful Φ range of the gradient, which is where log k' is in the range 1–0 (k' = 10–1), and then calculating the solvent strength values ($S = \Delta \log k'/\Delta\Phi$), determining the gradient steepness according to the Snyder linear solvent strength theory[27,28]. In order to do this by using only TLC R_M data, a method is proposed which consists in constructing R_M *vs.* Φ plots such as those reported in Fig. 4. From these plots gradient elution conditions were determined and compared with data obtained by HPLC (see Table VI). It can be seen that the agreement is generally satisfactory for the useful $\Delta\Phi$ range, whereas more significant differences were found in solvent strength evaluation. The latter failure is not critical, however, because gradient steepness must be often optimized in order to achieve optimum separations[13,14].

CONCLUSIONS

The correlation between HPLC and TLC is a very important experimental factor which can be of great value for the rapid and economical development of chromatographic methods for flavonoid analysis. However, the significant differences observed here suggest that care is necessary when using unvalidated data. The fact

TABLE VI

USEFUL Φ RANGES FOR GRADIENT ELUTION AND MEAN SOLVENT STRENGTH VALUES CALCULATED FROM LOG k' OR R_M DATA
Methanol as organic modifier. Acetic acid as acid modifier. HPLC data taken from ref. 16.

Compound	*HPLC*			*HPTLC (M)*			*TLC (M)*			*TLC (W)*		
	Φ $(k'=10)$	Φ $(k'=1)$	S	Φ $(k'=10)$	Φ $(k'=1)$	S	Φ $(k'=10)$	Φ $(k'=1)$	S	Φ $(k'=10)$	Φ $(k'=1)$	S
Eriodictyol	39	55	6.2	30	62	3.1	37	63	3.8	38	60	4.5
Naringenin	34	61	3.7	36	67	3.2	45	61	6.2	46	64	5.5
Acacetin	49	82	3.0	51	72	4.8	53	75	4.5	53	70	5.9
Apigenin	46	74	3.6	51	72	4.8	53	75	4.5	53	70	5.9
Apigenin 7G	36	54	5.5	35	64	3.4	38	61	4.3	40	59	5.3
Apiin	37	53	6.3	35	62	3.7	36	60	4.2	39	58	5.3
Chrysin	56	80	4.2	–	–	–	–	–	–	–	–	–
Chrysoeriol	45	72	3.7	51	72	4.8	54	78	4.2	54	73	5.3
Luteolin	43	65	4.5	45	71	3.8	49	69	5.0	48	66	5.5
Luteolin 7G	33	52	5.3	31	66	3.4	32	59	3.7	–	–	–
Galangin	54	80	3.8	54	75	4.8	60	81	4.8	60	81	4.8
Morin	36	58	4.5	–	–	–	–	–	–	–	–	–
Quercetagetin	28	45	5.9	–	–	–	–	–	–	–	–	–
Quercetin	42	59	5.9	41	70	3.4	46	69	4.3	45	65	5.0
Quercitrin	38	56	5.5	34	65	3.2	39	62	4.3	39	59	5.0
Rutin	34	52	5.5	29	60	3.2	33	58	4.0	35	56	4.8

that solvent selectivity effects previously described in HPLC are conserved in TLC makes the latter superior to HPLC owing to the possibility of enhancing the peak capacity through two-dimensional development[29]. This last point and its applications deserve a separate investigation.

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