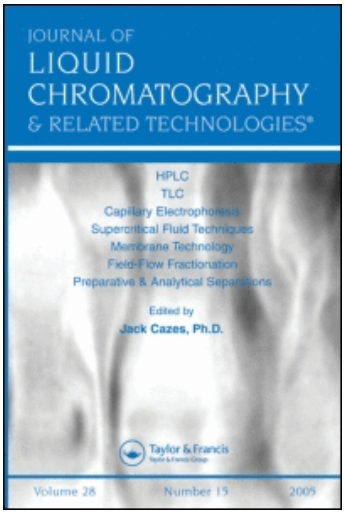

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EFFECT OF THE MOBILE AND STATIONARY PHASES ON RP-HPLC RETENTION AND SELECTIVITY OF FLAVONOID COMPOUNDS

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ABSTRACT

The retention behaviour of an extended set of flavonoid compounds on phenyl and cyano reversed-phase HPLC columns was studied and compared to behaviour on octadecyl column. The selectivity properties of methanol, acetonitrile and tetrahydrofuran as organic modifiers on each stationary phase are reported. Both the stationary and the mobile phases appeared to significantly change HPLC system selectivity. Specific stationary phase selectivity effects proved more pronounced with methanol: in particular the phenyl phase showed a greater selective retention for unsaturated flavonoids while octadecyl proved more selective for glycoside compounds.

INTRODUCTION

Flavonoid compounds occupy a prominent position among natural phenols, particularly due to their conspicuous presence in green plants as well as to their importance in the flavour and nutritional quality of foodstuffs. Since Reversed-Phase HPLC plays a central role in the separation of complex flavonoid mixtures, many systematic collections and investigations of

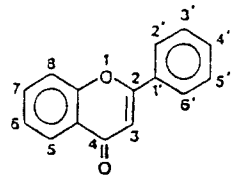
retention data have been published to date (1-3). The influence of the chromatographic variables (i. e., solvent, composition and type of stationary phase) in determining retention has been widely examined for an extended set of flavonoid compounds (4-8). Different solvents have exhibited significant selectivity features on octadecyl column; therefore an extended study of solvent effects on different stationary phases may lead to additional insights in retention selectivity. In the present paper, the specific selectivity effects of organic modifiers (tetrahydrofuran and acetonitrile) combined with different stationary phases (phenyl and cyano) is exploited and related to solute molecular structure. This study will be useful in setting up a multichoice chromatographic separation set and in selecting uncorrelated retention systems for optimum analytical separations.

MATERIALS AND METHODS

The chromatographic measurements were made according to experimental procedure previously reported (7, 8). The retention data, log k,' were evaluated with a Waters 600 multi-solvent system, equipped with Rheodyne injection valve (20μl sample loop), and a Waters 990 photodiode-array detector, coupled with an APC III personal computer (NEC). Two reversed phase columns were used: 30 cm x 3.9 mm, 10 μm Ph μBondapak Phenyl column (Waters) and 30 cm x 3.9 mm, 10 μm CN μBondapak Cyano column (Waters). On these columns log k' values were measured at a minimum of six different mobile phase compositions (expressed as ϕ %, i. e., organic solvent % proportions). Acetonitrile and tetrahydrofuran used as solvent were of HPLC grade (Carlo Erba, Milan, Italy) and water was purified by a Norganic System (Millipore, Bedford, MA, USA). The aqueous phase was buffered at pH2-3 in 80mM acetic acid/8mM disodium hydrogenphosphate, analytical reagent grade (Carlo Erba, Milan, Italy). The standards studied were from Sarsyntex (Merignac, France) and used as received. Standards (in methanol) had a concentration in the 10-100 ppm range. The set of flavonoid compounds selected, whose molecular structures are reported in Figure 1, represented the following classes: flavones, flavonols, flavanones and glycosides.

RESULTS AND DISCUSSION

The relationship between retention (log k') and mobile phase composition (ϕ) was calculated from the experimental data: the dependence of log k' on ϕ is linear for methanol and tetrahydrofuran, while it is parabolic for acetonitrile. These organic modifiers displayed the same behaviour as previously observed on the C-18 column (7). The best fit gave a correlation greater than 0.99 for all the systems examined: the correlation coefficients of the calculated equations are reported in Tables I-IV. The intercept value A (log k' value extrapolated to pure water), as a measure of the stationary phase retentivity independent of the organic modifier type, is a



No	Compound	3	5	7	2'	3'	4'
1	**Acacetin**		OH	OH			OMe
2	**Apigenin**		OH	OH			OH
3	**Apigenin 7-O-glucoside**		OH	O-Glu			OH
4	**Apiin**		OH	2Ap-Glu			OH
5	**Chrysin**		OH	OH		OH	OH
6	**Chryseriol**		OH	OH		OMe	OH
7	**Eriodictyol**		OH	OH			
8	**Galangin**	OH	OH	OH			
9	**Luteolin**		OH	OH		OH	OH
10	**Luteolin 7-O-glucoside**		OH	O-Glu		OH	OH
11	**Morin**	OH	OH	OH	OH		OH
12	**Naringenin**		OH	OH			OH
13	**Quercetin**	OH	OH	OH		OH	OH
14	**Quercetrin**	O-Rhm	OH	OH		OH	OH
15	**Rutin**	O-Rut	OH	OH		OH	OH
16	**Flavanone**						
17	**Flavone**						
18	**Flavanol**	OH					

Figure 1: Molecular structure of the flavonoid compounds studied.

TABLE I.
log k' vs. ϕ Parabolic Fitting according to the Equation:
$\log k' = A + B\,\phi + C\,\phi^2$
Organic Modifier: Acetonitrile; Column: Phenyl.
R = correlation coefficient; σ_{xy} = standard error of the regression.

	Compound	A	B	C	R	σ_{xy}
1	Acacetin	3.18±0.04	-8.24±0.20	5.00±0.22	1.00	0.01
2	Apigenin	2.86±0.11	-9.48±0.61	7.50±0.81	1.00	0.01
3	Apigenin 7-O-glucoside	2.55±0.02	-15.33±0.19	17.59±0.34	1.00	0.01
4	Apiin	2.39±0.04	-14.38±0.36	15.99±0.65	1.00	0.01
5	Chrysin	2.86±0.04	-7.16±0.20	4.00±0.22	1.00	0.01
6	Chrysoeriol	2.88±0.20	-9.40±1.14	7.36±1.50	0.99	0.01
7	Eriodictyol	2.58±0.08	-7.90±0.47	6.30+0.66	1.00	0.02
8	Galangin	2.97±0.02	-7.45±0.10	4.25±0.11	1.00	0.01
9	Luteolin	2.76±0.17	-10.47±1.03	9.37±1.45	0.99	0.03
10	Luteolin 7-O-glucoside	2.76±0.15	-15.47±1.16	18.46±2.07	1.00	0.01
11	Morin	2.68±0.01	-11.69±0.11	12.33±0.19	1.00	0.01
12	Naringenin	2.58±0.18	-7.93±1.05	5.79±1.39	0.99	0.01
13	Quercetin	2.44±0.11	-8.78±0.67	7.34±0.94	1.00	0.02
14	Quercitrin	2.76±0.03	-14.55±0.25	17.29±0.45	1.00	0.01
15	Rutin	2.55±0.14	-15.56±1.08	19.75±1.93	0.99	0.03
16	Flavanone	3.17±0.11	-7.12±0.85	3.75±0.56	1.00	0.01
17	Flavone	2.89±0.04	-7.31±0.20	4.50±0.22	1.00	0.01
18	Flavanol	3.04±0.06	-7.33±0.30	4.25±0.33	1.00	0.01

TABLE II.
log k' vs. ϕ Linear Fitting according to the Equation:
$\log k' = A + B\phi$
Organic Modifier: Tetrahydrofuran; Column: Phenyl.
R = correlation coefficient; σ_{xy}= standard error of the regression.

	Compound	A	B	R	σ_{xy}
1	Acacetin	2.51±0.20	-4.67+0.46	0.99	0.09
2	Apigenin	2.54±0.17	-4.97±0.41	0.99	0.10
3	Apigenin 7-O-glucoside	2.10±0.16	-5.52±0.54	0.99	0.08
4	Apiin	2.09±0.20	-5.90±0.67	0.99	0.10
5	Chrysin	2.45±0.18	-4.50±0.40	0.99	0.08
6	Chrysoeriol	2.15±0.16	-4.23±0.35	0.99	0.07
7	Eriodictyol	2.25±0.13	-4.13±0.29	0.99	0.06
8	Galangin	2.86±0.17	-5.06±0.38	0.99	0.07
9	Luteolin	2.34±0.15	-4.63±0.35	0.99	0.09
10	Luteolin 7-O-glucoside	2.12±0.20	-5.24±0.68	0.99	0.10
11	Morin	2.29±0.13	-4.60±0.30	0.99	0.08
12	Naringenin	2.41±0.14	-4.47±0.31	0.99	0.06
13	Quercetin	2.42±0.17	-4.60±0.3	0.99	0.07
14	Quercitrin	2.18±0.15	-5.22±0.50	0.99	0.07
15	Rutin	1.90±0.17	-4.63±0.56	0.99	0.08
16	Flavanone	2.26±0.13	-4.10±0.29	0.99	0.05
17	Flavone	1.90±0.13	-3.73±0.31	0.99	0.08
18	Flavanol	2.27±0.16	-4.07±0.36	0.99	0.07

TABLE III.
log k' vs. ϕ Parabolic Fitting according to the Equation:
$\log k' = A + B\phi + C\phi^2$
Organic Modifier: Acetonitrile; Column: Cyano.
R = correlation coefficient; σ_{xy} = standard error of the regression.

	Compound	A	B	C	R	σ_{xy}
1	Acacetin	2.22±0.11	-6.99±0.71	4.75±1.01	1.00	0.02
2	Apigenin	1.97±0.05	-7.33±0.41	5.76±0.66	1.00	0.02
3	Apigenin 7-O-glucoside	1.50±0.02	-7.53±0.22	5.73±0.54	1.00	0.01
4	Apiin	1.55±0.05	-8.14±0.57	5.91±1.40	1.00	0.01
5	Chrysin	2.08±0.06	-6.67±0.39	4.75±0.56	1.00	0.01
6	Chrysoeriol	2.04±0.05	-7.60±0.39	6.04±0.60	1.00	0.02
7	Eriodictyol	1.40±0.05	-5.92±0.47	5.38±0.91	1.00	0.01
8	Galangin	1.92±0.02	-5.54±0.28	3.25±0.11	1.00	0.01
9	Luteolin	1.83±0.09	-7.53±0.69	6.37±1.02	1.00	0.02
10	Luteolin 7-O-glucoside	1.53±0.10	-9.06±1.11	8.64±2.07	0.99	0.03
11	Morin	1.61±0.02	-7.41±0.21	7.20±0.41	1.00	0.01
12	Naringenin	1.47±0.05	-5.07±0.46	3.63±0.91	1.00	0.02
13	Quercetin	1.85±0.03	-8.16 ±0.33	8.17±0.66	1.00	0.01
14	Quercitrin	1.91±0.03	-8.79 ±1.06	9.09±1.58	0.99	0.02
15	Rutin	1.57±0.10	-14.57±1.08	21.81±1.97	0.99	0.03
16	Flavanone	1.83±0.04	-5.72±0.90	4.49±1.56	0.99	0.03
17	Flavone	1.83±0.04	-5.71±0.24	3.75±0.36	1.00	0.01
18	Flavonol	1.93±0.05	-5.67±0.31	3.50±0.43	1.00	0.01

TABLE IV.
log k' vs. ϕ Linear Fitting according to the Equation:
$\log k' = A + B\phi$
Organic Modifier: Tetrahydrofuran; Column: Cyano.
R = correlation coefficient; σ_{xy} = standard error of the regression.

	Compound	A	B	R	σ_{xy}
1	Acacetin	1.60±0.08	-4.49±0.21	0.99	0.02
2	Apigenin	1.61±0.07	-3.27±0.28	0.99	0.03
3	Apigenin 7-O-glucoside	1.24±0.05	-3.26±0.15	0.99	0.02
4	Apiin	1.19±0.04	-3.31±0.27	0.99	0.02
5	Chrysin	1.62±0.06	-4.58±0.15	0.99	0.01
6	Chrysoeriol	1.77±0.09	-4.10±0.19	0.99	0.02
7	Eriodictyol	1.61±0.06	-3.20±0.19	0.99	0.02
8	Galangin	1.76±0.07	-4.73±0.28	0.99	0.03
9	Luteolin	1.56±0.05	-3.59±0.15	0.99	0.01
10	Luteolin 7-O-glucoside	1.20±0.04	-3.27±0.18	0.99	0.01
11	Morin	1.53±0.04	-3.08±0.20	0.99	0.02
12	Naringenin	1.67±0.05	-3.22±0.17	0.99	0.02
13	Quercetin	1.55±0.04	-3.28±0.16	0.99	0.01
14	Quercitrin	1.31±0.03	-3.32±0.12	0.99	0.01
15	Rutin	1.17±0.03	-3.14±0.16	0.99	0.02
16	Flavanone	1.59±0.04	-3.97±0.19	0.99	0.02
17	Flavone	1.34±0.03	-4.00±0.11	0.99	0.01
18	Flavanol	1.52±0.03	-3.95±0.16	0.99	0.01

suitable parameter for comparing the different chromatographic systems. The experimental data obtained were related to those, previously reported, with methanol (MeOH) as solvent on the various stationary phases (8) and on the octadecyl (C-18) column with the different solvents (7). As stationary phase polarity increases and approaches that of the mobile phase, the stationary phase retentivity for each solvent decreases in the following order:

$$C\text{-}18 > Ph > CN.$$

The solvent strength S was computed in the interval between log k' = 1 and log k' = 0 and calculated as:

$$S = \Delta \log k' / \Delta\phi$$

The S values calculated for the different retention systems are reported in Table V. This parameter is very important for both isocratic and gradient elution to chose the best possible chromatographic conditions to optimize analytical elution (5, 7). The following order, as expected for solvent strength in RP-LC:

$$ACN > THF > MeOH$$

is not very clear for these data. A peculiar behaviour is shown by glycoside and aglycone classes. On the phenyl phase glycosides generally exhibit higher S values than do aglycones and, of the three solvents, the effect is most marked for acetonitrile. On the CN phase, methanol and tetrahydrofuran show the same solvent strength for both glycosides and aglycones, whereas when acetonitrile is the organic modifier glycosides show greater S values than do aglycones (see Table V). The same behaviour was previously observed for the C-18 phase. These results confirm the hypothesis previously assumed (8): specific interactions take place between the glycoside moiety and the CN group when acetonitrile is the bulk mobile phase, whereas there is no specific effect when CN is bonded to the stationary phase. On the basis of this information, the linear solvent strength (LSS) gradient theory developed by Snyder (9, 10) was useful in determining optimum gradient elution conditions for the separation of complex flavonoid mixtures.

The general pattern of the structure-retention relationship of these columns was studied by determining group contributions to retention (Δ log k') for various substituents in the benzopyran ring (i. e. the difference between retention of one molecule containing a particular substituent and that of a molecule which does not contain that group). Only experimental log k' data, roughly in the 0 - 1 range, were employed. Table VI reports mean Δ log k' values calculated from 4-5 different mobile phase compositions with their standard deviations. On the phenyl phase, a slight dependence of Δ log k' on ϕ is often observed in tetrahydrofuran and acetonitrile: more markedly in the case of glycosides. Similar behaviour had previously been observed on the C-18 phase (7). On the cyano phase, it can be seen that Δ log k' values are almost independent of mobile phase composition: the least retentive CN phase appears to be the least sensitive to changes in solute structure and solvent composition. Specific solute-stationary phase interactions on the different columns can be revealed by relating Δ log k' values for different column pairs. The resulting Δ log k' - Δ log k' correlations show good statistical coefficients (eqs. 1-9, Table VII).

TABLE V.
Mean Solvent Strength S of Methanol (MeOH), Acetonitrile (ACN) and Tetrahydrofuran (THF) on Phenyl and Cyano Columns.

	Compound	Phenyl column			Cyano column		
		MeOH	**ACN**	**THF**	**MeOH**	**ACN**	**THF**
1	Acacetin	4.8	3.3	4.3	4.8	3.7	4.0
2	Apigenin	5.0	3.8	4.7	3.7	4.2	3.9
3	Apigenin 7-O-glucoside	5.3	7.1	5.5	3.2	5.5	3.5
4	Apiin	5.3	7.1	6.6	3.4	6.2	3.7
5	Chrysin	4.5	3.5	4.2	4.0	3.6	3.8
6	Chryoeriol	5.0	3.7	4.5	4.0	4.2	4.0
7	Eriodictyol	4.3	3.7	4.2	2.4	3.6	2.9
8	Galangin	4.5	3.6	4.2	4.0	3.6	3.9
9	Luteolin	5.0	4.3	5.0	3.3	4.3	3.8
10	Luteolin 7-O-glucoside	5.3	8.3	5.9	3.2	6.2	3.8
11	Morin	5.0	4.7	5.0	2.9	4.5	3.1
12	Naringenin	4.3	3.7	4.3	2.9	3.3	3.1
13	Quercetin	5.0	4.0	4.7	3.2	4.3	3.3
14	Quercitrin	5.3	7.1	5.0	3.0	5.9	3.4
15	Rutin	5.3	7.3	4.3	3.0	8.3	3.9
16	Flavanone	4.2	3.5	3.7	3.3	2.7	3.1
17	Flavone	4.2	4.2	4.2	3.6	3.3	3.4
18	Flavanol	4.0	3.2	3.6	3.7	3.2	3.5
	Common mean and S. D.	4.8 ±0.5	4.7 ±1.7	4.7 ±0.8	3.4 ±0.6	4.5 ±1.4	3.6 ±0.4
	Aglycone mean and S. D.	4.6 ±0.4	3.7 ±0.4	4.3 ±0.4	3.6 ±0.6	3.7 ±0.5	3.5 ±0.4
	Glycoside mean and S. D.	5.3 ±0.0	7.3 ±0.5	5.7 ±0.6	3.2 ±0.2	6.4 ±1.1	3.7 ±0.2

TABLE VI.
Substituent Group Contributions to Retention (Δ log k')
with the Different Columns (Phenyl and Cyano) and Solvents (ACN and THF).
Data are reported as mean Δ log k' values with their standard deviations.

Group contribution	compounds	Phenyl ACN (0.2-0.4)*	Phenyl THF (0.3-0.5)*	Cyano ACN (0.1-0.4)*	Cyano THF (0.1-0.4)*
3-OH	1) 13-9	0.02±0.01	0.12±0.04	-0.02±0.02	0.08±0.01
	2) 18-17	0.08±0.03	0.23±0.07	0.08±0.02	0.19±0.02
	3) 8-5	0.02±0.02	0.16±0.06	0.03±0.03	0.11±0.01
3'-OH	4) 9-2	-0.20±0.05	-0.08±0.04	-0.13±0.03	-0.06±0.01
	5) 7-12	-0.22±0.04	-0.08±0.04	-0.15±0.02	-0.05±0.01
	6) 10-3	-0.21±0.04	-0.07±0.05	-0.14±0.03	-0.04±0.01
4'OH	7) 2-5	-0.35±0.05	-0.11±0.03	-0.20±0.01	-0.27±0.08
4'-OCH_3	8) 1-5	0.04±0.03	-0.02±0.02	0.03±0.04	-0.01±0.01
2, 3 unsaturation	9) 16-17	0.20±0.06	0.36±0.08	0.15±0.04	0.26±0.01
	10) 12-2	0.03±0.03	0.10±0.02	-0.04±0.08	0.07±0.01
	11) 7-9	0.03±0.01	0.08±0.02	-0.03±0.02	0.08±0.01
3-glycoside (rhamnose)	12) 14-13	-0.46±0.04	-0.53±0.11	-0.38±0.05	-0.33±0.01
3-glycoside (rutinose)	13) 15-13	-0.81±0.03	-0.80±0.18	-0.58±0.10	-0.44±0.02
7-glycoside (glucose)	14) 3-2	-0.65±0.06	-0.60±0.12	-0.48±0.05	-0.37±0.01
	15) 10-9	-0.66±0.03	-0.56±0.13	-0.47±0.09	-0.36±0.01
7-glycoside (apiosylglucose)	16) 4-2	-0.78±0.16	-0.74±0.12	-0.55±0.08	-0.43±0.01

* Mobile phase composition range.

TABLE VII.
Statistic Coefficients of Correlations between Mean Δ log k' Data Obtained for Different Stationary Phase and Modifier Pairs:
$\Delta \log k'_1 = A + B \Delta \log k'_2$.
R = correlation coefficient; $\sigma_{y.x}$ = standard error of the regression.
Data for methanol taken from Refs. 7 and 8.

Stationary phase pairs (1, 2)	Modifier	A	B	R	$\sigma_{y.x}$	Eq.
Phenyl-C-18	MeOH	-0.05±0.02	0.61±0.07	0.926	0.07	(1)
	ACN	-0.01±0.01	0.91±0.03	0.991	0.05	(2)
	THF	-0.03±0.02	0.95±0.05	0.982	0.06	(3)
Cyano-C-18	MeOH	-0.01±0.04	0.67±0.09	0.881	0.10	(4)
	ACN	-0.01±0.01	0.64±0.03	0.988	0.04	(5)
	THF	-0.01±0.01	0.62±0.03	0.984	0.04	(6)
Cyano-Phenyl	MeOH	0.02±0.04	0.99±0.16	0.854	0.11	(7)
	ACN	-0.01±0.01	0.71±0.02	0.992	0.03	(8)
	THF	0.01±0.02	0.62±0.05	0.954	0.07	(9)
Modifier pair (1, 2)	Column	A	B	R	$\sigma_{y.x}$	Eq.
ACN - MeOH	C-18	0.05±0.07	1.13±0.19	0.840	0.20	(10)
	Ph	0.12±0.06	1.71±0.21	0.906	0.15	(11)
	CN	0.02±0.02	1.04±0.13	0.907	0.10	(12)
THF - MeOH	C-18	0.13±0.08	1.06±0.23	0.781	0.24	(13)
	Ph	0.18±0.08	1.65±0.30	0.823	0.21	(14)
	CN	0.08±0.05	0.93±0.17	0.821	0.14	(15)
THF - ACN	C-18	0.10±0.02	0.99±0.05	0.980	0.07	(16)
	Ph	0.08±0.03	1.03±0.07	0.966	0.10	(17)
	CN	0.07±0.02	0.95±0.06	0.970	0.06	(18)

These equations appear particularly significant because they relate phenyl and cyano systems to the octadecyl column, whose retention behaviour has been widely described (4-8).

The intercepts obtained were near 0. For the pair phenyl - C-18 the slope values were near 1 when ACN and THF were the organic modifiers (eqs. 2, 3). According to Melander classification (11), this behaviour suggests a *homoenergetic* retention: identical retention energies are involved in the elution on the two stationary phases. When the retention values on the cyano phase were related to those on C-18 (eqs. 4-6), the slope values of 0.6 prove that the corresponding Gibbs energy for the two columns is not identical at a fixed temperature, rather it is proportional (*homeoenergetic* binding) (11): on the more polar CN phase, the retention energy is less than is found on the less polar C-18. A similar equation was obtained when Δ log k' - Δ log k' correlation was calculated for the pair Ph - C-18 when methanol was the organic modifier (eq. 1). This behaviour as *homeoenergetic* retention due to the solvent, can most likely be explained by considering that the solvent molecules participate with the stationary phase in determining solute retention. In fact, various retention models, describing the solute distribution between the mobile phase and the solvent-surface stationary phase, assume the stationary phase to be a thin layer of solvent, the composition of which is determined via molecular interactions with the solid support (12-15).

When the experimental Δ log k' values are compared with the calculated values (according to eqs. 1-6, Table VII) the resulting differences may measure the retention specificity of the given retention systems (14). Figures 2 and 3, reporting the difference values ($\Delta \log k'_{exp.} - \Delta \log k'_{calc.}$) for each substituent group, show that the most pronounced deviations, and therefore the strongest selectivity effects, were displayed by methanol as compared with acetonitrile and tetrahydrofuran. In particular, the stronger selectivity of the phenyl phase towards unsaturate compounds (higher positive deviations for substituent groups 9-11) was shown as well as the distinct specific retention of the C-18 phase towards the glycoside compounds (higher negative differences for substituents 12-14, 16). The positive deviations of unsatured compounds may be attributed to the increased specific interactions of these aromatic, more highly conjugated molecules with the phenyl groups of the stationary phase (16, 18). The dependence on the nature of the organic modifier may be explained on the basis of the existence of strong interactions (such as polar and hydrogen bonding) between the OH group of the adsorbed methanol and the polar solute molecules, also containing OH groups. Moreover, due to the restructuring of the stationary phase to form relatively ordered alkyl groups containing solvent molecules, the stationary phase seems to be more ordered in methanol than in other solvents, since methanol molecules are known to associate with one another by hydrogen bonding.

This peculiarity of methanol as organic modifier was further emphasized by the correlation between solvent pairs on each column (eqs. 10-18 in Table VII). The THF-ACN pair (eqs. 16-18) exhibited the best statistic parameters, intercept value near 0 and slope near 1. On the other hand, when acetonitrile and tetrahydrofuran were related to methanol (eqs. 10-15) the statistical quality of the correlation worsened and slope was at times different from 1. This means that

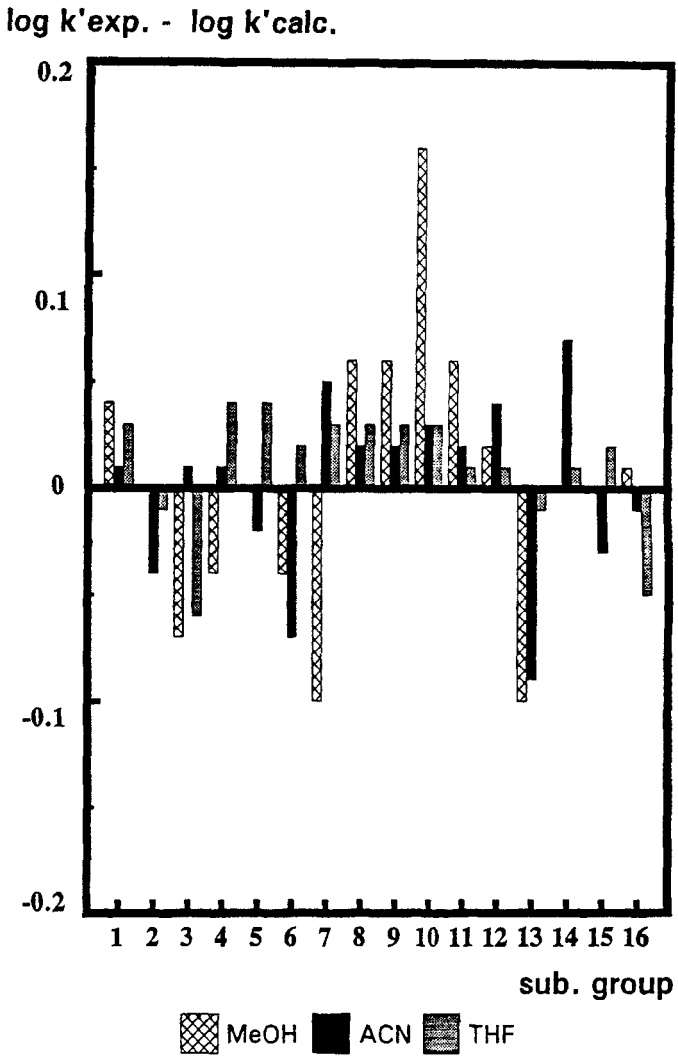


Figure 2: $\Delta \log k'_{experimental} - \Delta \log k'_{calculated}$ difference values for various substituent groups calculated from the $\Delta \log k' - \Delta \log k'$ correlation for the phenyl - C-18 column pair (eqs. 1-3 in Table VII).
Number of group contributions as in Table VI.

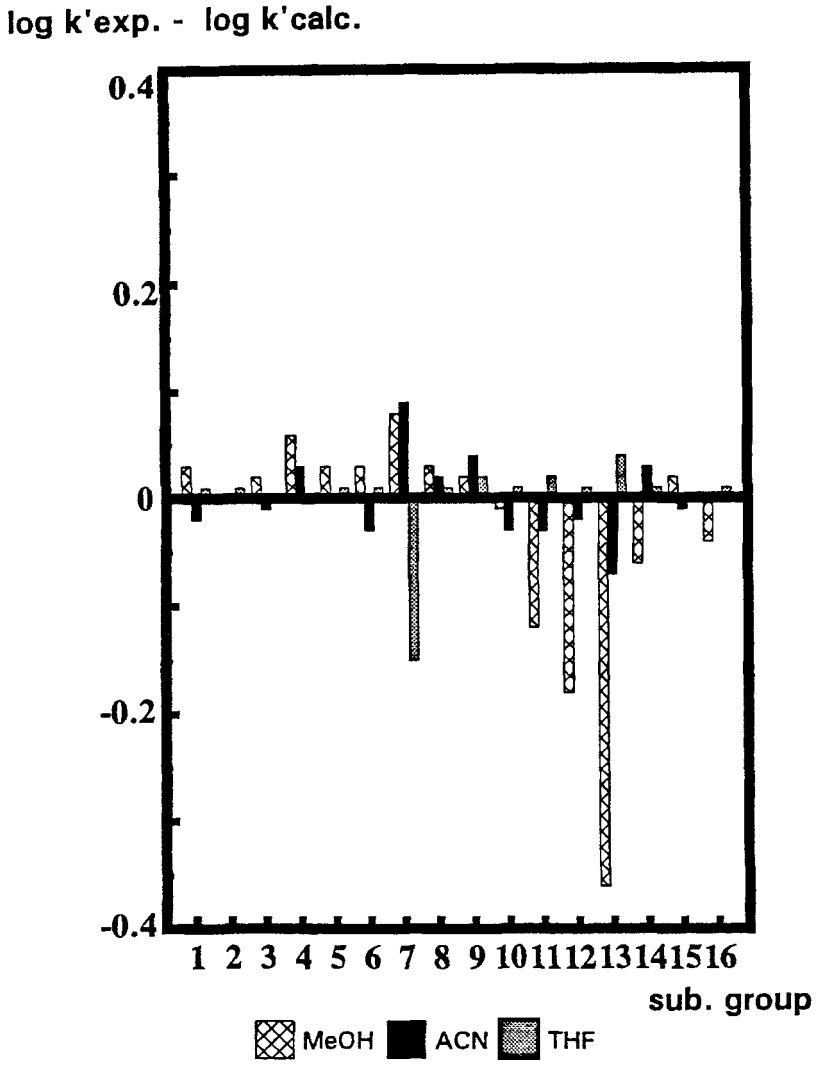


Figure 3: Δ log k'$_{experimental}$ - Δ log k'$_{calculated}$ difference values for various substituent groups calculated from the Δ log k' - Δ log k' correlation for the cyano - C-18 column pair (eqs. 4-6 in Table VII).
Number of group contributions as in Table VI.

pairing methanol elution with tetrahydrofuran and acetonitrile elutions, can provide two poorly correlated elution systems. This behaviour, valid on each column, is especially strong for the C-18 column.

CONCLUSIONS

This comparative study of retention properties of different RP chromatographic systems for flavonoid compounds made it possible to single out specific selectivity effects and their dependence on solute molecular structure. Detailed analysis of the selectivity of different column-solvent pairs would appear to be the guide-line for the choice of the most useful RP system to solve separation problems, when poorly correlated retention systems are required. In fact, when a complex mixture containing many flavonoid compounds is to be analyzed, overlapping retention is most likely on a single retention system, while the use of different, poorly correlated column-mobile phase different systems can reduce overlapping and improve detection and quantitation.

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