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PREDICTION OF RETENTION BEHAVIOUR OF SOME PHENOLIC ANTI-OXIDANTS

M. POPL, I. VÍT and F. ŠMEJKAL

Department of Analytical Chemistry, Institute of Chemical Technology, Suchbátarova 5, 166 28 Prague 6 (Czechoslovakia)

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SUMMARY

The possibility of the prediction of retention behaviour was studied in reversed-phase liquid chromatography on a macroporous organic gel (Separon SE) with methanol–water as eluent. Linear dependences were found in the effect of the mobile phase composition on the retention of all the solutes tested. For the prediction of retention data in a binary mobile phase, the slope of the straight line (k) and the y intercept (log I_0) must be known. The chromatographic system was tested with several standards, and from these data the Δk and $\Delta \log I_0$ contributions were calculated. The contributions then served for calculation of the retention indices of phenolic antioxidants for any composition of the binary mobile phase.

INTRODUCTION

A significant task in chromatography is prediction of the retention behaviour of the separated components. This problem has been the concern of a number of workers, aiming to determine the retention of the components either by calculation from some experimental chromatographic data or from other physical parameters of the compounds. Empirical or semi-empirical equations have been frequently used. Sleight[1], for instance, expressed the chromatographic behaviour of parent and substituted aromatic hydrocarbons in a Zipax Permaphase ODS–methanol–water system by the simple equation log $k' = A + BC'$, where k' is the capacity factor, C' is the number of carbon atoms in an aromatic hydrocarbon or the total number of alkyl carbons, and A and B are constants. Similar dependences have been found also in reversed-phase partition liquid chromatography (RP-LLC) on macroporous organic gels[2].

In the study of the separation mechanism in RP-LLC, the solubility of the solute in the mobile phase constituted by water and some suitable organic solvent has been found to be of significance. Considerable attention has been paid to the relation between the chromatographic behaviour of the components and their solubility in water, and surprisingly good results have been obtained. Locke[3,4] has demonstrated that for chemically related components, their relative retentions can be determined

from the differences in their solubility in the mobile phase. The contributions of the substituents to the retention of the molecule have been studied in detail, and the sums of the contributions from the various substituents related to the solubility[5,6].

Hermann[7] has found a relation between the calculated surface area of the water cavity and the solubility of hydrocarbons in water; analogous results have been obtained by Amidon et al.[8] for an alcohol series.

The topological index of molecular connectivity, κ, has also been employed for prediction of the retention. This quantity is calculated from the structure of the substance in question and some of its physical parameters, such as the solubility in water, the boiling point, or the cavity surface area[9,10]. Karger et al.[11] have used this index to predict the retention of substituted phenols and cyclic alcohols.

Tijssen et al.[12] have made use of the solubility parameters of the substance and the stationary and mobile phases for a prediction of the retention; based on these parameters, the authors define the distribution constant of the substance, from which they are able to determine the retention. They achieved good results in gas–liquid chromatography; in liquid chromatography the prediction was limited because of lack of accurate data for the orientation and transfer interactions.

The extent to which the above methods of prediction of the retention are warranted has been dealt with by Horváth et al.[13], who offer a detailed analysis of the factors responsible for the retention of the components in a RP-LLC system.

The aim of the present work is to suggest a novel method for predicting the retention in RP-LLC based on some chromatographic data, and in the case of some rather complex compounds to demonstrate the agreement between the calculated and experimental data.

EXPERIMENTAL

The measurements were carried out on a stainless-steel column (250 × 6 mm I.D.), fitted with an injection head packed with spherical beads, 25–30 μm diameter, of the organic copolymer Separon SE with the exclusion limit of mol.wt. 300,000 (Laboratorní přístroje, Prague, Czechoslovakia).

The eluent was delivered by means of a VLD 30 high-pressure linear syringe pump (Development Workshop, Czechoslovak Academy of Sciences, Prague, Czechoslovakia). The detection was accomplished by means of a UVD 5 detector at 254 nm, using a 10-μl cell with a path length of 1 cm (Development Workshop, Czechoslovak Academy of Sciences).

Methanol–water linear mixtures served as the mobile phase; the flow-rate was 60 ml/h.

The chromatography standards were reagent grade purity. Commercial phenolic antioxidants were used (Table I) without further purification.

RESULTS AND DISCUSSION

We have previously examined the retention behaviour of aromatic hydrocarbons[2], their derivatives with polar functional groups[14], and aromatic nitro compounds[15] on the Separon SE polystyrene macroporous gel. This substance has been shown to behave in RP-LLC in a way similar to, e.g., Zipax Permaphase ODS or

TABLE I

LIST OF ANTIOXIDANTS

Trade name	Company	Compound
Desoxon M	Synthesia, Pardubice Czechoslovakia	4,4'-Methylenebis-(2,6-dimethylphenol)
AOX 4K	CHZJD, Bratislava Czechoslovakia	2,6-Di-tert.-butyl-p-cresol
Vulkanox NKF	Bayer, Leverkusen, G.F.R.	1,1-Bis-(2-hydroxy-3,5-dimethylphenyl)-isobutane
Vulkanox BKF	Bayer	2,2'-Methylenebis-(4-methyl-6-tert.-butylphenol)
Topanol CA	ICI, Macclesfield, Great Britain	1,1,3-Tris-(2-methyl-5-tert.-butyl-4-hydroxyphenyl)-butane

some related sorbent. The effects of the methanol–water, acetonitrile–water and tetrahydrofuran–water mobile phases and of the non-polar or polar substituents on the retention of the molecule have been examined. In the papers cited, the results have been presented in the form of the retention indices[16], which are as follows: by definition, $\log I = 1$, 2, and 3 for benzene, naphthalene, and phenanthrene, respectively, and for the separed components the retention indices are calculated as

$$\log I = \log I_n + \frac{\log V'_x - \log V'_n}{\log V'_{n+1} - \log V'_n}$$

where the V' values are the reduced retention volumes, and the n, $n + 1$, and x subscripts refer to the nearest lower and higher standards and the separated solute, respectively.

Moreover, it has been suggested that the retention indices of polysubstituted compounds can be calculated as the sum of the contributions ($\Delta\log I$) from the functional groups bonded on the aromatic system. The $\Delta\log I$ increments are the differences of the values for the monosubstituted benzene derivatives and benzene in the mobile phase used. In this manner, the retention behaviour can be determined for the mobile phase in which the increment values have been measured. A shortcoming of this procedure is the fact that it is limited to the composition of the mobile phase that was used for determining the increment values. The accuracy of the prediction reduced considerably if the substituents in the molecule interact with one another.

Linear dependences were found in the effect of the mobile phase composition on the retention of alkylbenzenes and alkylphenols for methanol–water systems and the Separon SE sorbent (Figs. 1–4). As follows from the data obtained, the change in the retention index in dependence on the mobile phase composition can be described by the simple equation:

$$\log I = k(\%, \text{v/v, methanol}) + \log I_0$$

where k is the slope of the straight line and $\log I_0$ is the y intercept.

There are some trends in the slopes for various compounds.

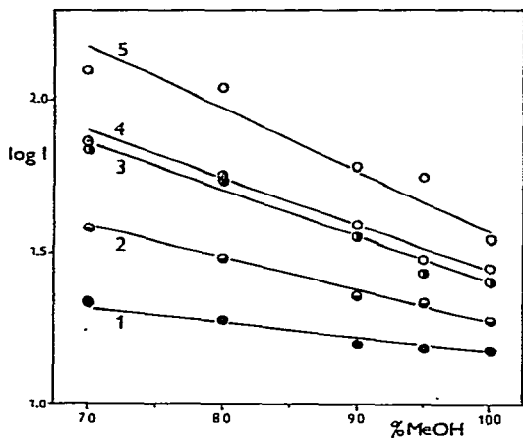(1) Their absolute values for n-alkyl derivatives increases with increasing

Fig. 1. Dependence of retention indices of *n*-alkylbenzenes on the composition of the mobile phase. Mobile phase: methanol–water. Stationary phase: Separon SE. 1 = Methylbenzene; 2 = ethylbenzene; 3 = propylbenzene; 4 = 1,3,5-trimethylbenzene; 5 = butylbenzene. MeOH = Methanol.
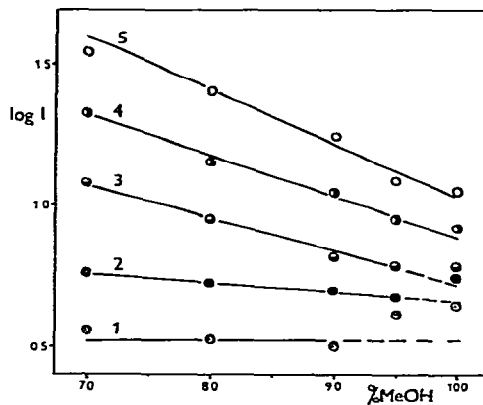
Fig. 2. Dependence of retention indices of *n*-alkylphenols on the composition of the mobile phase. Mobile phase: methanol–water. Stationary phase: Separon SE. 1 = Phenol; 2 = *p*-methylphenol; 3 = *p*-ethylphenol; 4 = *p*-propylphenol; 5 = *p*-butylphenol.
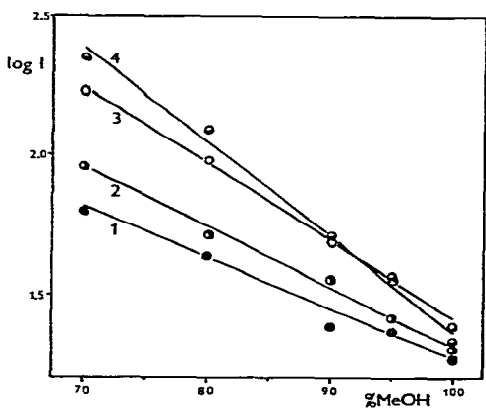

Fig. 3. Dependence of retention indices of branched alkylbenzenes on the composition of the mobile phase. Mobile phase: methanol–water. Stationary phase: Separon SE. 1 = Isopropylbenzene; 2 = *tert.*-butylbenzene; 3 = *tert.*-amylbenzene; 4 = *m*-diisopropylbenzene.
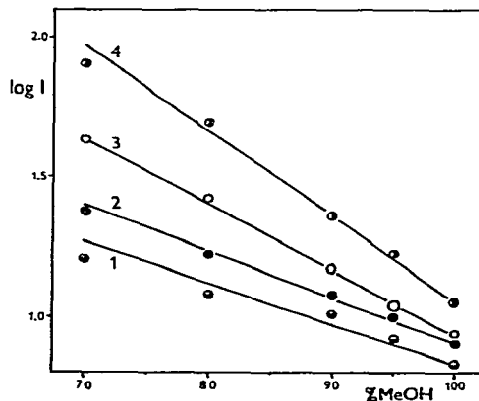
Fig. 4. Dependence of retention indices of branched alkylphenols on the composition of the mobile phase. Mobile phase: methanol–water. Stationary phase: Separon SE. 1 = *p*-Isopropylphenol; 2 = *o*-isopropylphenol; 3 = *p*-tert.*-amylphenol; 4 = 2,6-di-isopropylphenol.

number of carbon atoms in the chain: the increase is linear, the Δk contribution for a methylene group is -0.005.

(2) The absolute values of the slope increase if the alkyl group is branched, the effect being as follows: (a) a tertiary carbon atom increases the slope in such a manner that the value is the same as that of the linear chain with one additional carbon atom;

(b) a secondary carbon atom increases the absolute slope values to a lesser extent than a tertiary carbon; the effect can be described by the surplus value of -0.002.

(3) If a higher number of alkyl substituents are present in the molecule, their effect on the slope is additive.

The above rules can be illustraded by the following examples of prediction of the slopes:

n-propylbenzene $3 \cdot (-0.005) = -0.015$
$tert$.-amylbenzene $5 \cdot (-0.005) + 1 \cdot (-0.005) = -0.030$.

The contributions from alkyl, secondary and tertiary carbonatoms in an alkyl substituent to the slopes, Δk, for the separated solutes are given in Table II. It is of significance that the contributions are the same for akylbenzenes and alkylphenols.

TABLE II

GROUP CONTRIBUTIONS FOR CALCULATION OF THE SLOPE (Δk) AND y INTERCEPT ($\Delta \log I_0$)

Group	Δk	$\Delta \log I_0$
sec. C	-0.002	—
tert. C	-0.005	—
alkyl C	-0.005	$+0.65$
aromatic ring	—	$+1.00$
hydroxyl group	—	-0.50

Calculation of the slopes requires the knowledge of the contributions only from the alkyl substituents, not from the aromatic systems. This is a consequence of the fact that in the calculations the retention indices are used, and as stated above, those for benzene, naphthalene, and phenanthrene are assigned constant values, $\log I = 1$, 2, and 3, respectively; hence, the slope is zero for them.

For a prediction of the retention index in a binary mobile phase of a chosen composition, not only the slope, but also the y intercept must be known in the $\log I = f(\%$, v/v, methanol) linear relation. The intercept, $\log I_0$, represents the retention index of the solute that would be observed if water was used as the mobile phase. The extrapolated $\log I_0$ values were obtained by correlation analysis, and the results compare favourably with the increment theory. In the series of substances measured, the $\log I_0$ value depends on the number of aromatic rings, number of hydroxyl groups, and total number of carbon atoms in the alkyl substituent, and is independent of the structure of the alkyl substituent. The values of $\Delta \log I_0$ contributions are given in Table II. For instance, for p-isopropylphenol the $\log I_0$ value can be calculated as

$$\log I_0 = 1 \cdot (+1) + 3 \cdot (+0.65) + 1 \cdot (-0.050) = +2.45$$

The predicted $\log I_0$ values could not be verified experimentally, because in pure water as the mobile phase the retention volumes are too high.

TABLE III

MEASURED AND CALCULATED VALUES OF LOG I FOR DIFFERENT CONTENT OF METHANOL IN THE MOBILE PHASE

Antioxidant	Correlation coefficient	Slope		Intercept		Methanol (% v/v)	log I		D
		Meas.	Calc.	Meas.	Calc.		Meas.	Calc.	
Desoxon M	−0.991	−0.021	−0.025	3.81	4.25	70	2.34	2.50	−0.16
						80	2.22	2.25	−0.03
						90	1.94	2.00	−0.06
						95	1.84	1.88	−0.04
						100	1.76	1.75	+0.01
AOX 4K	−0.994	−0.050	−0.055	6.23	6.35	80	2.19	1.95	+0.24
						90	1.69	1.40	+0.29
						95	1.51	1.13	+0.39
						100	1.15	0.85	+0.30
Vulkanox NKF	−1.000	−0.052	−0.044	6.52	6.20	80	2.33	2.68	−0.35
						90	1.82	2.24	−0.42
						95	1.56	2.02	−0.46
						100	1.28	1.80	−0.52
Vulkanox BKF	−0.995	−0.078	−0.065	9.37	8.15	80	3.07	2.95	+0.12
						90	2.33	2.30	+0.03
						95	2.01	1.98	+0.03
						100	1.46	1.65	−0.19
Topanol CA	−0.999	−0.111	−0.114	11.70	13.85	80	2.79	4.73	−1.94
						90	1.71	3.59	−1.88
						95	1.22	3.02	−1.82
						100	0.54	2.45	−1.91

For practical purposes, the importance of the method is in the possibility of predicting the retention indices for substances with complex structures. A prerequisite is the use of the same stationary phase (column) and the mobile phase with the same binary eluent as those employed in the tests.

The applicability of this method to the prediction of the retention indices of the separated substances was tested in the separation of phenolic antioxidants. The predicted and measured values are given in Table III. The log I_{calc} values were calculated by using the basic contributions given in Table II. For instance, for Topanol CA the slope was calculated to be

$$19 \cdot (-0.005) + 3 \cdot (-0.005) + 2 \cdot (-0.002) = -0.114$$

as against the experimental value of -0.111.

Although the antioxidants are substances with complex structures, the measured and calculated retention index values are in good agreement. Topanol CA is an exception, possibly owing to the mutual influence of the substituents in the molecule. Since the measured values for this solute are considerably lower than the calculated ones, it can be assumed that owing to the steric arrangement, the hydrophobic nature of the molecule is lower than would correspond to the presence of the *tert.*-butyl substituents and, particularly, of the sterically shielded main butyl chain.

Within the studied region of composition of the methanol–water mobile phases, the log $I = f$(%, v/v, methanol) function for the antioxidants is linear (Fig. 5).
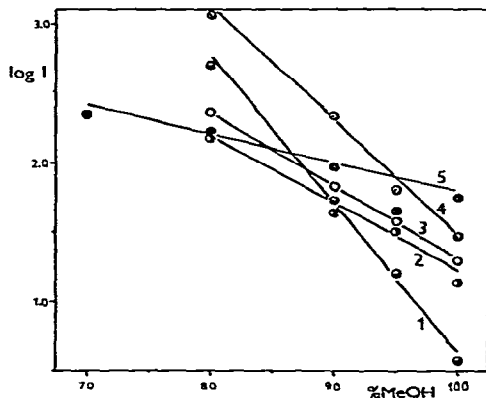

Fig. 5. Dependence of retention indices of phenolic antioxidants on the composition of the mobile phase. Mobile phase: methanol–water. Stationary phase: Separon SE. 1 = Topanol CA; 2 = AOX 4K; 3 = Vulkanox NKF; 4 = Vulkanox BKF; 5 = Desoxon M.

For methanol contents in the mobile phase below 70% (v/v), the antioxidants are markedly retarded by the column. Changes in the order of the eluted solutes on varying the methanol content in the mobile phase have been observed with polyalkylbenzenes and -phenols, notably when the alkyl substituents were branched or contained more than three carbon atoms. In chromatographic separation in RP-LLC systems, such substances give a steep dependence of the log I values on the composition of the mobile phase. This can be explained similarly to the manner in which

Scott and Simpson[17] explain the properties of sorbents with chemically bonded phases in aqueous mobile phases. Owing to the polar nature of the aqueous mobile phase, the dispersion forces can effect a spatial arrangement of the molecule such that the volume of the molecule is reduced. With its increasing content in the mobile phase, methanol penetrates within the substituents of the molecule, which thus get unfolded (the methanol–substituent dispersion interaction is commensurable with the substituent–substituent interactions). From the point of view of the separation mechanism, there obviously are two factors operating. Owing to the unfolding of the alkyl substituents, the hydrophobic nature of the molecule increases, and as a consequence, the retention should be higher. In the opposite sense, the better solvation of the molecule by methanol, results in a higher affinity for the mobile phase. In this respect the volume of the molecule, which increases with the methanol content in the mobile phase, is of great importance, as it is in gel permeation chromatography.

CONCLUSIONS

It has been demonstrated that the retention behaviour in RP-LLC of components with rather complex molecules can be predicted fairly well, even when the composition of the mobile phase varies. As a prerequisite, the chromatographic system has to be tested with several basic standards. Based on the results thus obtained, the Δk and $\Delta \log I_0$ contributions (Table II) are calculated. Since the $\Delta \log I_0$ contributions refer to extrapolations to pure water, they should apply also the chromatographic systems other than that dealt with in this work. The Δk and $\Delta \log I_0$ contributions can than serve for calculation of the retention indices of complex molecules for any composition of the binary mobile phase. In this manner, it should be possible to predict any changes in the order of the eluting components, and hence in the selectivity of the mobile phase.

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