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## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC BEHAVIOUR OF N-SUBSTITUTED PHTHALIMIDES

### CALCULATION OF CAPACITY FACTORS FOR MEMBERS OF THE HOMOLOGOUS SERIES\*

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#### SUMMARY

The high-performance liquid chromatographic behaviour of some N-substituted phthalimides on normal and reversed-phase columns was studied. The retention of the compounds is discussed in terms of shielding of the polar  $-\text{CO}-\text{N}-\text{CO}-$  group of phthalimide by non-polar or slightly polar substituents. The linear dependence of  $\log k'$  on the number of carbon atoms of the alkyl substituent of N-alkylphthalimides has been used as a basis for deriving a general formula for calculating the capacity factor for any member in the homologous series.

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#### INTRODUCTION

Our recent investigation of the kinetics and mechanism of the reaction of hydroxy compounds with phthalimide in the presence of triphenylphosphine and diethyl azodicarboxylate<sup>1,2</sup> required the use of a fast and precise analytical method. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) seemed to be suitable for this purpose. However, the chromatographic separation of N-substituted phthalimides had not been described, except for the GC separation of a few chloroethyl phthalimide compounds<sup>3</sup>.

We have endeavoured to develop a suitable separation method; because of the very high boiling point of phthalimide and its derivatives, HPLC was assumed to be more suitable than GC.

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\* Part of a Ph.D. Thesis, P. Dufek, Charles University, Prague, 1982.

## EXPERIMENTAL

N-Substituted phthalimides were prepared by the reaction of phthalimide with an appropriate hydroxy compound in the presence of triphenylphosphine and diethyl azodicarboxylate by a slight modification of the method of Mitsunobu and co-workers<sup>4,5</sup>. The compounds were identified by mass (MS) and nuclear magnetic resonance (NMR) spectrometry, or by the conformity of their retention times with those of standards obtained either commercially or by the conventional Gabriel synthesis.

The measurements were performed on a Varian Model 4100 liquid chromatograph equipped with a Variscan 635 UV detector, or on a Spectra-Physics SP 8700 liquid chromatograph equipped with a UVM-4 UV detector (Development Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia). The wavelength selected for all measurements was 290 nm. The calculations were performed using a Varian Chromatography Data System CDS 111.

Stainless-steel columns containing LiChrosorb 10 RP-18 and 10 RP-8 (10  $\mu\text{m}$ , 250  $\times$  4.6 mm), Hi-Eff C<sub>18</sub> (5  $\mu\text{m}$ , 250  $\times$  5.3 mm) and Separon Si C<sub>18</sub> (10  $\mu\text{m}$ , 250  $\times$  6.0 mm) were used with a mobile phase of methanol–water (50:50 to 80:20, v/v) at a flow-rate of 2.0–4.0 ml/min. Some measurements were carried out on a LiChrosorb Si-60-7 column (7  $\mu\text{m}$ , 250  $\times$  4.6 mm) using heptane–2-propanol (92:8 and 94:6, v/v) as mobile phase at 5.0 ml/min.

## RESULTS AND DISCUSSION

The retention data of 43 N-substituted phthalimides were measured on various columns using various mobile phase compositions.

The chromatographic properties of these derivatives on a reversed-phase column are determined by two factors. (1) The shielding of the polar  $-\text{CO}-\text{N}-\text{CO}-$  group of phthalimide by a non-polar or a slightly polar substituent. This results in weaker interactions between this polar group and the polar mobile phase (methanol–water). Similar shielding was observed in the case of *s*-triazines<sup>6,7</sup>. (2) A non-polar substituent at the N-atom of phthalimide increases the hydrophobic character of the molecule and the interactions between the substituent and the non-polar stationary phase (octadecane, octane) become stronger. The more non-polar the substituent, the stronger is the retention of the compound by the column.

On polar stationary phases such as silica gel the above interactions, as well as the elution order, are reversed.

The effect of the two factors is reflected in the retention data and capacity factors of N-alkylphthalimides on LiChrosorb RP-18 for the four compositions of the mobile phase (Table I). The longer the carbon chain of the alkyl group, the higher are the values of retention time and capacity factor. This is due to a more effective shielding of the polar phthalimide group and the more hydrophobic character of the molecule.

N-Alkylphthalimides with a branched or cyclic alkyl group, e.g., N-(3-methylbutyl)phthalimide and N-cyclohexylphthalimide, and those with an unsaturated alkyl group, e.g., N-allylphthalimide, are less strongly retained on the column than saturated isomers with a non-branched carbon chain. The only exception is N-(1-methylethyl)phthalimide which is eluted after N-propylphthalimide, and can be

TABLE I  
RETENTION DATA OF N-ALKYLPHthalIMIDES

Column: LiChrosorb RP-18. Mobile phase: methanol-water, 2.0 ml/min. UV detection at 290 nm.

| Alkyl group    | Methanol-water ratio |       |             |       |             |       |             |       |
|----------------|----------------------|-------|-------------|-------|-------------|-------|-------------|-------|
|                | 50:50                |       | 60:40       |       | 70:30       |       | 80:20       |       |
|                | $t_R$ (min)          | $k'$  | $t_R$ (min) | $k'$  | $t_R$ (min) | $k'$  | $t_R$ (min) | $k'$  |
| H              | 2.60                 | 0.60  | 2.04        | 0.26  | 1.76        | 0.08  | 1.68        | 0.03  |
| Methyl         | 4.19                 | 1.57  | 2.85        | 0.75  | 2.24        | 0.37  | 1.90        | 0.17  |
| Ethyl          | 6.98                 | 3.28  | 4.03        | 1.46  | 2.76        | 0.69  | 2.16        | 0.33  |
| Propyl         | 12.21                | 6.49  | 6.00        | 2.68  | 3.53        | 1.17  | 2.49        | 0.53  |
| Butyl          | 24.71                | 14.15 | 10.10       | 5.20  | 4.96        | 2.04  | 3.04        | 0.87  |
| Pentyl         | 50.28                | 29.85 | 17.63       | 9.82  | 7.24        | 3.44  | 3.82        | 1.34  |
| Hexyl          | —                    | —     | 31.97       | 18.61 | 11.07       | 5.79  | 5.00        | 2.07  |
| Heptyl         | —                    | —     | —           | —     | 17.71       | 9.87  | 6.83        | 3.19  |
| Octyl          | —                    | —     | —           | —     | 29.24       | 16.94 | 9.56        | 4.87  |
| Nonyl          | —                    | —     | —           | —     | 48.01       | 28.45 | 13.71       | 7.41  |
| Decyl          | —                    | —     | —           | —     | —           | —     | 19.95       | 11.24 |
| Undecyl        | —                    | —     | —           | —     | —           | —     | 29.55       | 17.13 |
| 1-Methylethyl  | —                    | —     | 6.19        | 2.80  | 3.61        | 1.21  | 2.59        | 0.59  |
| 2-Methylpropyl | —                    | —     | 8.94        | 4.48  | 4.59        | 1.82  | 2.96        | 0.82  |
| 3-Methylbutyl  | —                    | —     | —           | —     | 6.90        | 3.23  | 3.75        | 1.30  |
| Cyclohexyl     | —                    | —     | 20.39       | 11.51 | 8.74        | 4.36  | 4.38        | 1.69  |
| Allyl          | —                    | —     | 4.23        | 1.60  | 2.83        | 0.74  | 2.18        | 0.34  |
| 2-Butenyl*     | —                    | —     | 7.06        | 3.33  | 3.81        | 1.34  | 2.64        | 0.62  |
| —              | —                    | —     | 7.70        | 3.72  | 4.04        | 1.48  | 2.64        | 0.62  |
| 3-Butenyl      | —                    | —     | 6.42        | 2.94  | —           | —     | —           | —     |
| Benzyl         | —                    | —     | 9.83        | 5.03  | 4.69        | 1.88  | 2.91        | 0.79  |

\* *cis*- and *trans*-isomers.

accounted for by a less effective shielding due to the steric arrangement of the alkyls. Furthermore, in the case of derivatives with unsaturated alkyl groups an interaction can occur between their  $\pi$ -electrons and the polar mobile phase, which results in a further decrease in the capacity factors, *e.g.*, for N-benzylphthalimide.

A plot of  $\log k'$  vs. number of carbon atoms is linear for N-alkylphthalimides with non-branched alkyl chains, with the exception of the first members of the homologous series, *i.e.*, phthalimide, N-methyl- and sometimes also N-ethyl- and N-propylphthalimides, which are less strongly retained on the column than would be expected (see Fig. 1).

The location of the double bond also plays a significant rôle, *cf.*, *cis*- and *trans*-N-(2-butenyl)phthalimides and N-(3-butenyl)phthalimide. This is again caused by ineffective shielding of the polar group of phthalimide by small alkyl groups. The deviations from linearity are more significant with increasing percentage of methanol in the mobile phase. A chromatogram of a mixture of N-alkylphthalimides is shown in Fig. 2.

The linear dependence of  $\log k'$  on the number of carbon atoms of the alkyl residue can be used to calculate the capacity factor of any member of the homologous

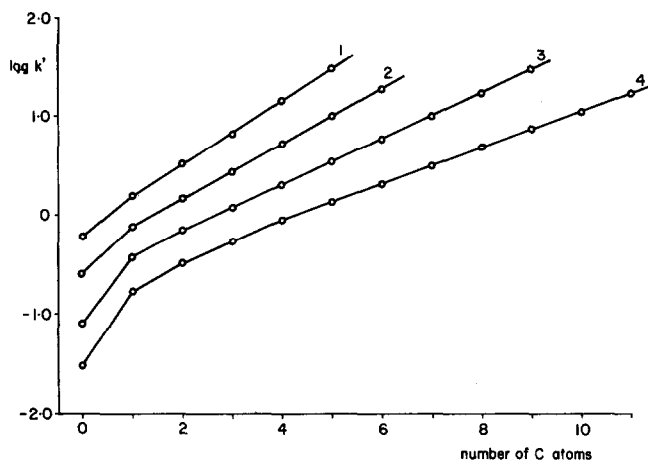


Fig. 1. Dependence of  $\log k'$  on the number of carbon atoms in the alkyl groups for N-alkylphthalimides with a non-branched carbon chain. Column: LiChrosorb RP-18. Mobile phase: methanol-water in the ratios 50:50 (1), 60:40 (2), 70:30 (3), 80:20 (v/v) (4).

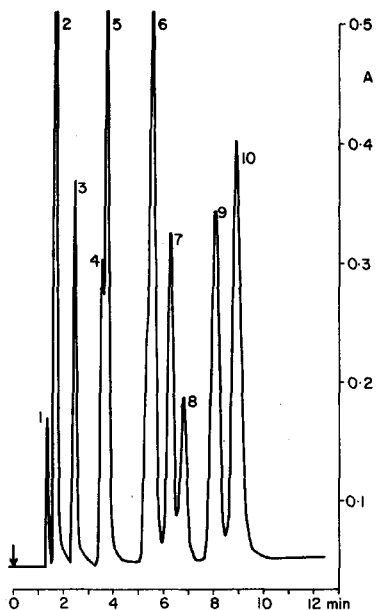


Fig. 2. Separation of a mixture of N-alkylphthalimides on LiChrosorb RP-18 with methanol-water (60:40 v/v) as mobile phase. Flow-rate 2.0 ml/min. UV detection at 290 nm. Peaks: 1 = solvent; 2 = phthalimide; 3 = N-methylphthalimide; 4 = N-ethylphthalimide; 5 = N-allylphthalimide; 6 = N-propyl- and N-(1-methylethyl)phthalimide; 7,8 = *cis*- and *trans*-N-(2-butenyl)phthalimides; 9 = N-(2-methylpropyl)phthalimide; 10 = N-butylphthalimide.

series under the same chromatographic conditions, *e.g.*, column, mobile phase composition, temperature, etc.

$$\log k'_{n+1} - \log k'_n = \log \frac{k'_{n+1}}{k'_n} = \text{constant} = K \quad (1)$$

$$\frac{k'_{n+2}}{k'_n} = \frac{k'_{n+1}}{k'_n} \cdot \frac{k'_{n+2}}{k'_{n+1}} = K^2 \quad k'_{n+2} = k'_n K^2 \quad (2)$$

⋮  
⋮  
⋮

$$\frac{k'_{n+i}}{k'_n} = \frac{k'_{n+i}}{k'_{n+i-1}} \cdot \frac{k'_{n+i-1}}{k'_{n+i-2}} \cdots \frac{k'_{n+1}}{k'_n} = K^i, \text{ hence} \quad (3)$$

$$k'_{n+i} = k'_n K^i, \quad K = \sqrt[i]{k'_{n+i}/k'_n} \quad (4)$$

where  $k'_n$  is the capacity factor of a homologue containing  $n$  carbon atoms in the alkyl group,  $i$  is the difference in the number of carbon atoms in the alkyl chain between homologues containing  $n$  and  $n + i$  carbon atoms and  $K$  is a factor reflecting the change in the capacity factor of a homologue when the carbon chain is extended or shortened by a single  $-\text{CH}_2-$  group. Extension occurs when  $i \geq 1$ ; when  $i \leq -1$  the carbon chain is shortened.

The factor  $K$  can be calculated according to eqn. 4 using known values of the capacity factors of two homologues containing  $n$  and  $n + i$  carbon atoms. The

TABLE II

COMPARISON OF MEASURED AND CALCULATED CAPACITY FACTORS FOR N-ALKYLPHTHALIMIDES ON LICHROSORB RP-18 WITH METHANOL-WATER MOBILE PHASES

| Alkyl   | Methanol-water ratio |       |       |       |       |       |       |       |
|---------|----------------------|-------|-------|-------|-------|-------|-------|-------|
|         | 50:50                |       | 60:40 |       | 70:30 |       | 80:20 |       |
|         | Meas.                | Calc. | Meas. | Calc. | Meas. | Calc. | Meas. | Calc. |
| Methyl  | 1.57                 | 1.55  | 0.75  | 0.76  | 0.37  | 0.42  | 0.17  | 0.24  |
| Ethyl   | 3.28                 | 3.24  | 1.46  | 1.44  | 0.69  | 0.71  | 0.33  | 0.37  |
| Propyl  | 6.49                 | 6.78  | 2.68  | 2.74  | 1.17  | 1.20  | 0.53  | 0.57  |
| Butyl   | 14.15                | —     | 5.20  | —     | 2.04  | —     | 0.87  | —     |
| Pentyl  | 29.85                | 29.59 | 9.82  | 9.88  | 3.44  | 3.47  | 1.34  | 1.33  |
| Hexyl   | —                    | 61.85 | 18.61 | 18.77 | 5.79  | 5.90  | 2.07  | 2.04  |
| Heptyl  | —                    | —     | —     | 35.67 | 9.87  | 10.02 | 3.19  | 3.12  |
| Octyl   | —                    | —     | —     | 67.77 | 16.94 | 17.04 | 4.87  | 4.77  |
| Nonyl   | —                    | —     | —     | —     | 28.45 | 28.97 | 7.41  | 7.29  |
| Decyl   | —                    | —     | —     | —     | —     | 49.24 | 11.24 | 11.16 |
| Undecyl | —                    | —     | —     | —     | —     | —     | 17.13 | 17.07 |

TABLE III

## RETENTION DATA OF N-(HYDROXYALKYL)PHTHALIMIDES AND DIPHthalIMIDOALKANES

Chromatographic conditions: A, LiChrosorb RP-18, methanol-water (70:30), 2.0 ml/min; B, LiChrosorb RP-8, methanol-water (60:40), 2.0 ml/min; C, LiChrosorb Si-60-7, heptane-2-propanol (92:8), 5.0 ml/min; D, LiChrosorb Si-60-7, heptane-2-propanol (94:6), 5.0 ml/min. UV detection at 290 nm.

| Compound                           | A              |      | B              |      | C              |      | D              |      |
|------------------------------------|----------------|------|----------------|------|----------------|------|----------------|------|
|                                    | $t_R$<br>(min) | $k'$ | $t_R$<br>(min) | $k'$ | $t_R$<br>(min) | $k'$ | $t_R$<br>(min) | $k'$ |
| Diphthalimidomethane               | 2.45           | 0.50 | —              | —    | 1.88           | 1.94 | 2.34           | 2.66 |
| 1,2-Diphthalimidoethane            | —              | —    | —              | —    | 1.77           | 1.77 | 2.06           | 2.22 |
| 1,3-Diphthalimidopropane           | —              | —    | —              | —    | 1.61           | 1.52 | 1.87           | 1.92 |
| 1,4-Diphthalimidobutane            | 4.19           | 1.57 | 7.22           | 3.43 | 1.50           | 1.34 | 1.68           | 1.63 |
| 1,5-Diphthalimidopentane           | 5.96           | 2.66 | 10.55          | 5.47 | 1.42           | 1.22 | 1.58           | 1.47 |
| 1,6-Diphthalimidohexane            | 7.53           | 3.62 | 16.64          | 9.21 | 1.32           | 1.06 | 1.47           | 1.29 |
| 1,3-Diphthalimidobutane            | 3.50           | 1.15 | —              | —    | 1.41           | 1.20 | 1.59           | 1.58 |
| 1,4-Diphthalimido-2-butene         | 5.01           | 2.07 | —              | —    | 1.39           | 1.17 | 1.53           | 1.48 |
| N-(4-Hydroxybutyl)phthalimide      | 1.91           | 0.17 | 2.39           | 0.47 | 3.21           | 4.02 | 4.61           | 6.20 |
| N-(5-Hydroxypentyl)phthalimide     | 2.16           | 0.33 | 3.00           | 0.84 | 2.89           | 3.52 | 4.07           | 5.36 |
| N-(6-Hydroxyhexyl)phthalimide      | 2.55           | 0.56 | 3.94           | 1.42 | 2.55           | 2.98 | 3.51           | 4.48 |
| N-(3-Hydroxybutyl)phthalimide      | 2.02           | 0.24 | —              | —    | 1.97           | 2.08 | 2.54           | 3.17 |
| N-(4-Hydroxy-2-butenyl)phthalimide | 1.96           | 0.20 | —              | —    | 2.58           | 3.03 | 3.43           | 4.65 |
| Phthalimide                        | 1.76           | 0.08 | 2.03           | 0.25 | 1.21           | 0.75 | 1.30           | 1.03 |

TABLE IV

## RETENTION DATA OF N-SUBSTITUTED PHTHALIMIDES

Column: Separon Si C<sub>18</sub>. Mobile phase: methanol-water (70:30, v/v), 3.0 ml/min.

| Compound                       | $t_R$<br>(min) | $k'$ | $K$         |
|--------------------------------|----------------|------|-------------|
| Phthalimide                    | 1.91           | 1.01 |             |
| N-Methylphthalimide            | 2.34           | 1.46 |             |
| N-Ethylphthalimide             | 2.75           | 1.89 |             |
| N-Propylphthalimide            | 3.34           | 2.52 |             |
| N-Butylphthalimide             | 4.30           | 3.53 |             |
| N-Pentylphthalimide            | 5.75           | 5.05 | 1.38 ± 0.06 |
| N-(4-Hydroxybutyl)phthalimide  | 2.04           | 1.15 |             |
| N-(5-Hydroxypentyl)phthalimide | 2.26           | 1.38 |             |
| N-(6-Hydroxyhexyl)phthalimide  | 2.54           | 1.67 | 1.21 ± 0.01 |
| 1,2-Diphthalimidoethane        | 2.38           | 1.51 |             |
| 1,3-Diphthalimidopropane       | 3.00           | 2.16 |             |
| 1,4-Diphthalimidobutane        | 3.72           | 2.92 |             |
| 1,5-Diphthalimidopentane       | 4.24           | 3.46 |             |
| 1,6-Diphthalimidohexane        | 5.96           | 5.27 | 1.37 ± 0.14 |
| Methyl 2-phthalimidoacetate    | 2.05           | 1.16 |             |
| Methyl 2-phthalimidopropionate | 2.45           | 1.58 |             |
| Methyl 2-phthalimidobutyrate   | 2.75           | 1.89 |             |
| Methyl 2-phthalimidovalerate   | 3.20           | 2.37 | 1.27 ± 0.08 |
| Methyl 3-phthalimidopropionate | 2.15           | 1.26 |             |
| N-(2-Chloroethyl)phthalimide   | 2.75           | 1.89 |             |
| N-(2-Methoxyethyl)phthalimide  | 2.32           | 1.44 |             |
| N-(2-Ethoxyethyl)phthalimide   | 2.59           | 1.73 |             |
| 3-Phthalimidopropionitrile     | 2.73           | 1.87 |             |
| 3-Phthalimidonorbornamide      | 1.72           | 0.81 |             |

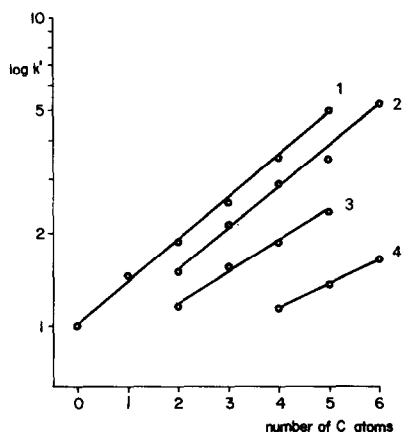


Fig. 3. Dependence of  $\log k'$  on the number of carbon atoms of the substituent bonded to the N-atom of phthalimide. Column: Separon Si  $C_{18}$ . Mobile phase: methanol-water (70:30 v/v), flow-rate 3.0 ml/min. UV detection at 290 nm. Curves: 1, N-alkylphthalimides; 2, 1, $\omega$ -diphthalimidoalkanes; 3, methyl esters of 2-phthalimidocarboxylic acids; 4, N-( $\omega$ -hydroxyalkyl)phthalimides.

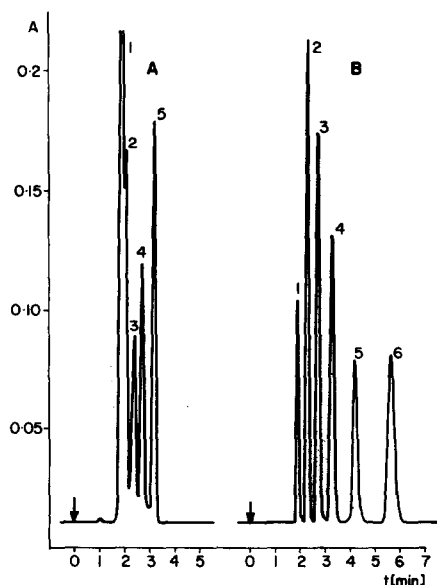


Fig. 4. Separation of mixtures of phthalimide derivatives on Separon Si  $C_{18}$  with methanol-water (70:30 v/v) as mobile phase, flow-rate 3.0 ml/min. UV detection at 290 nm. Peaks: A, 1 = phthalimide; 2 = methyl 2-phthalimidoacetate; 3 = methyl 2-phthalimidopropionate; 4 = methyl 2-phthalimidobutyrate; 5 = methyl 2-phthalimidovalerate; B, 1 = phthalimide; 2 = N-methylphthalimide; 3 = N-ethylphthalimide; 4 = N-propylphthalimide; 5 = N-butylphthalimide; 6 = N-pentylphthalimide.

capacity factor,  $k'_{n+j}$ , of a homologue containing  $n + j$  carbon atoms is then calculated according to:

$$k'_{n+j} = k'_n K^j$$

This equation can be used for calculating the capacity factor of any homologue of any series, assuming the dependence of  $\log k'$  vs. the number of structural units is linear.

The constant  $K$  for N-alkylphthalimides with non-branched carbon chains chromatographed on a LiChrosorb RP-18 column using methanol-water as mobile phase was calculated using the data given in Table I:  $K_{50} = 2.09 \pm 0.08$ ;  $K_{60} = 1.90 \pm 0.04$ ;  $K_{70} = 1.70 \pm 0.02$ ;  $K_{80} = 1.53 \pm 0.01$ . The subscript gives the percentage of methanol in the mobile phase. The  $k'$  values of non-linear homologues were not considered in the calculation.

A comparison of the measured and calculated capacity factors for N-alkylphthalimides is given in Table II. The  $k'$  values were calculated using the above  $K$  values and  $k'$  values of N-butylphthalimide as a reference compound. The error does not exceed 2% with the exception of the first non-linear homologues.

The chromatographic behaviour of phthalimide derivatives which carry alkyls further substituted by another functional group is dependent not only on the length of the alkyl carbon chain, but also on the polarity of the substituent group. The chromatographic data are summarized in Tables III and IV.

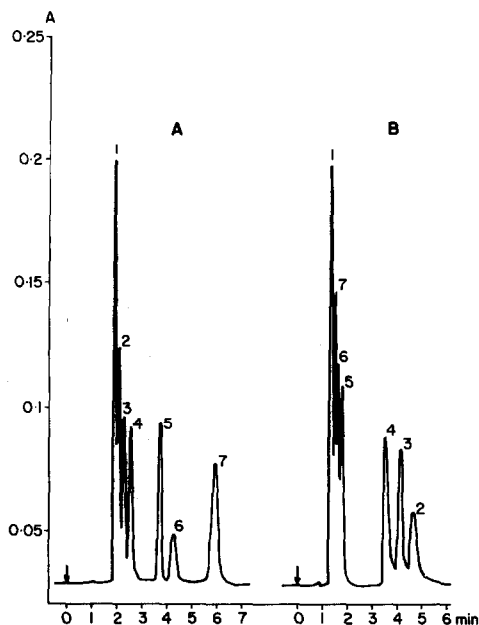


Fig. 5. Separation of a mixture of phthalimide derivatives. A, Column: Separon Si C<sub>18</sub>. Mobile phase: methanol-water (70:30 v/v), 3.0 ml/min. UV detection at 290 nm. B, Column: LiChrosorb Si-60-7. Mobile phase: heptane-2-propanol (96:4 v/v), 5.0 ml/min. UV detection at 290 nm. Peaks: 1 = phthalimide; 2 = N-(4-hydroxybutyl)phthalimide; 3 = N-(5-hydroxypentyl)phthalimide; 4 = N-(6-hydroxyhexyl)phthalimide; 5 = 1,4-diphthalimidobutane; 6 = 1,5-diphthalimidopentane; 7 = 1,6-diphthalimidohexane.

The dependence of retention on the Separon Si C<sub>18</sub> column on the polarity of the substituent was investigated for a series of N-ethylphthalimides substituted in position 2 of the ethyl group, C<sub>6</sub>H<sub>4</sub>(CO)<sub>2</sub>N-CH<sub>2</sub>CH<sub>2</sub>-R. The capacity factors decrease in the sequence: R = -CH<sub>3</sub> > -H ≥ -Cl ≥ -CN > -OC<sub>2</sub>H<sub>5</sub> > -COOCH<sub>3</sub> > -OCH<sub>3</sub> > -CONH<sub>2</sub> ≥ -OH. The substituent polarity increases in the same order. The *k'* value when R = OH, i.e., for N-(2-hydroxyethyl)phthalimide, was estimated by extrapolation of curve 4 in Fig. 3 (*k'* ≈ 0.8).

Fig. 3 shows the dependence of log *k'* on the number of carbon atoms for N-alkylphthalimides, diphthalimidoalkanes, methyl esters of 2-phthalimidocarboxylic acids and N-(hydroxyalkyl)phthalimides on a column packed with Separon Si C<sub>18</sub>. Since this dependence is linear for all the series it is possible to calculate the *K* values (see Table IV), according to eqn. 4. It can be seen that the above elution and polarity sequence is valid also for other homologues.

The separation of different types of phthalimide derivatives on Separon Si C<sub>18</sub> and LiChrosorb Si-60-7 columns is demonstrated in Figs. 4 and 5.

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