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RETENTION INDEX SCALE FOR LIQUID–LIQUID CHROMATOGRAPHY

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SUMMARY

A retention index scale based on the relative retention of a homologous series of C_3–C_{23} 2-keto alkanes was used to characterize the chromatographic properties of several drugs with four basic reversed-phase systems each using several mobile phase compositions. The retention index of a given drug was found to be fairly independent of exact composition of the mobile phase. Small changes in the retention index were observed for some drugs following a change in column type and these index changes could be related to changes in selectivity of the solvent–column system.

INTRODUCTION

One of the major problems in the utilization of high-pressure liquid chromatographic (HPLC) data is the comparison of retention time data from different literature reports. For a particular family of compounds, the narcotics for example, one will find the retention times for the same compound will differ because of the variety of the column-solvent systems used. Though the reproducibility is greatly improved when relative retention times are used, there is little uniformity in the selection of reference compounds and therefore it is still difficult to compare data from different sources.

If a large number of compounds of widely varying polarity are chromatographed, it is usually necessary to use several different solvent systems each with its own reference compound to establish a relative retention time scale. Because of this, a given compound will frequently have two relative retention times given in the same literature report. More frequently however, the overlap of two relative retention time scales is not detected or made evident in the report.

In the area of gas–liquid chromatography (GLC), the Kováts retention index scale has become widely used to standardize the reporting of GLC data and as a tool for the correlation of GLC properties and chemical structure[1,2]. The Kováts index of a given compound is determined by measuring the retention time of the compound and two *n*-paraffins that bracket the compound. By definition, methane would have a Kováts index of 100 and ethane, 200. The Kováts index of the test compound would then be found by interpolation between the values for the *n*-paraffins.

The major objective of the work described in this report was to investigate the possibility of constructing a retention index scale based on the retention of 2-keto alkanes that would be useful for HPLC analysis. Bonded, reversed-phase columns were investigated the most extensively because of their wide use in drug analysis and because it was anticipated that the chromatographic properties of the compounds would be more predictable than with liquid–solid chromatography.

EXPERIMENTAL

Materials

All of the columns used were 30 cm × 3.9 mm I.D. and contained packings with a 10-μm particle size. The packings used were a bonded octadecyl reversed-phase (μBondapak C_{18}; Waters Assoc., Milford, Mass., U.S.A.), a bonded cyanoalkyl reversed-phase (μBondapak CN, Waters Assoc.) and a silica adsorption packing (μPorasil, Waters Assoc.).

The C_3–C_{23} 2-keto alkanes were obtained from Analabs (North Haven, Conn., U.S.A.). The majority of the drug standards were obtained from Theta (Media, Pa., U.S.A.). The methanol and acetonitrile were freshly distilled. All other chemicals were of reagent-grade and were used without further purification.

Chromatography

All of the reversed-phase systems were used with a 0.025 *M* NaH_2PO_4 buffer that had been adjusted to an apparent pH of 7.0 after the methanol or acetonitrile had been added. In the case of the 90% methanol and the 70% acetonitrile systems, the NaH_2PO_4 content was reduced to 0.01 *M* because of the poor solubility of the salts. The flow-rate was 2.0 ml/min for all of the chromatographic systems.

A Waters Assoc. Model 202 chromatograph equipped with a U6K injector and a Model M-6000 pump was used for the study. Dual ultraviolet detectors operating at 254 and 280 nm were also used.

Measurement of retention indices

The capacity factor (k') of the drugs and standards were determined from the observed retention time (t_R) and the retention time of the solvent front (t_0) using the equation below:

$$k' = \frac{t_R - t_0}{t_0}$$

The retention index of a given 2-keto alkane standard was by definition equal to 100 times the number of carbons in the compound. Thus, acetone was assigned a value of 300 and 2-butanone, 400. The retention index (I) of a given drug or other test compound was calculated from the observed capacity factor for the drug (k'_D), the capacity factor for a 2-keto alkane eluting just before the test compound (k'_N), and the capacity factor of the next higher homologous (k'_{N+1}) using the equation below:

$$I = 100 \frac{\log k'_D - \log k'_N}{\log k'_{N+1} - \log k'_N} + 100\,N$$

As an alternative, the retention index of the test compound can be determined from a semi-log plot of capacity factor and the retention index of the ketone standards (Fig. 1). From the measured k' value of the test compound, its retention index could be determined by graphical interpolation of the standards. Though this method is slightly less precise, it is often convenient for many applications.

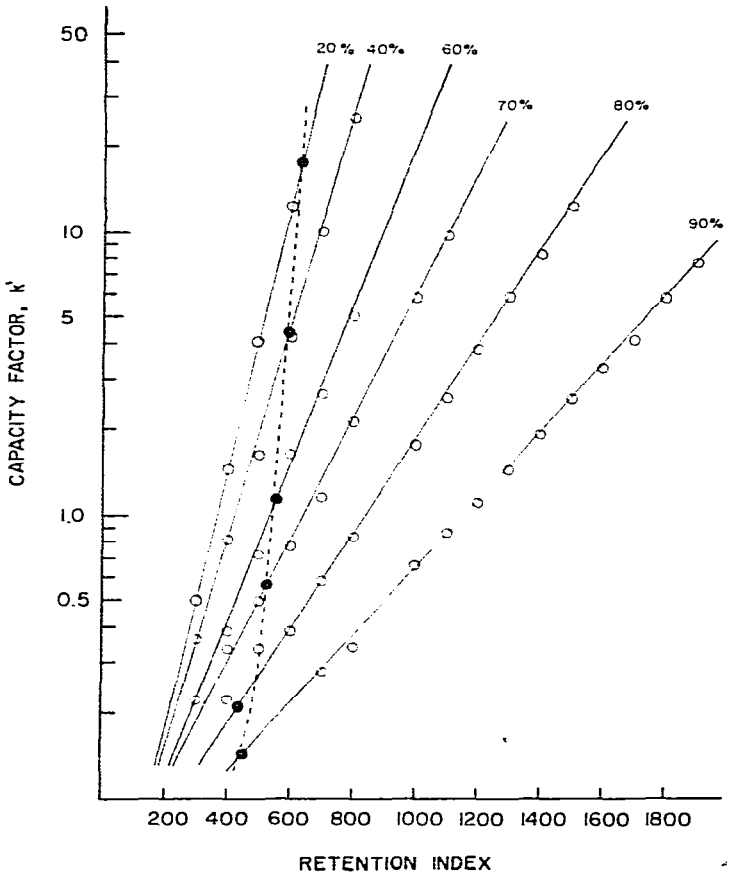

Fig. 1. Effect of solvent composition on capacity factor. ○, 2-Keto alkanes; ●, phenacetin, μBondapak C_{18} column, pH 7.0, 0.025 M NaH_2PO_4 aqueous buffer with varying methanol content.

RESULTS AND DISCUSSION

In general it was found that the plots of log k' *vs.* retention index were linear over a very wide range of conditions (Fig. 1). As shown below, the capacity factor is proportional to the equilibrium partition ratio (K) when the volumes of the stationary phase (V_s) and mobile phase (V_m) are taken into account[3]. When 20% methanol was used in the mobile phase with the μBondapak

$$k' = K\frac{V_s}{V_m}$$

C_{18} column (Fig. 1), the log k' value increased 0.42 units per methylene group. As the methanol content increased the slope of the curves and thus the change in log k'/CH_2 was found to decrease. Using the Hansch analysis method[4], the value for the logarithm of the octanol–water partition coefficient would be expected to increase 0.50 units for each methylene group. If the slopes of the family of curves in Fig. 1 were extrapolated to 0% methanol, a value of 0.48 was found for the increase in log k'/CH_2. In a previous study of the retention of aliphatic carboxylic acids and alcohols on a μBondapak C_{18} column, it was found that the increase in log k'/CH_2 was 0.502 and 0.509, respectively when no alcohol was used in the mobile phase[5]. With the carboxylic acids and alcohols, it was also found that slopes of the curves decrease and the intercepts of the curves remained approximately the same when the methanol content in the mobile phase was increased.

From these findings one could conclude that the change in free energy associated with the addition of one methylene group was nearly identical for the octanol–water partition system, the μBondapak C_{18}–water chromatographic system, and the μBondapak CN–water chromatographic system. The free energy change also appeared to be the same regardless if the terminal group on the alkyl chain was a hydrocarbon,

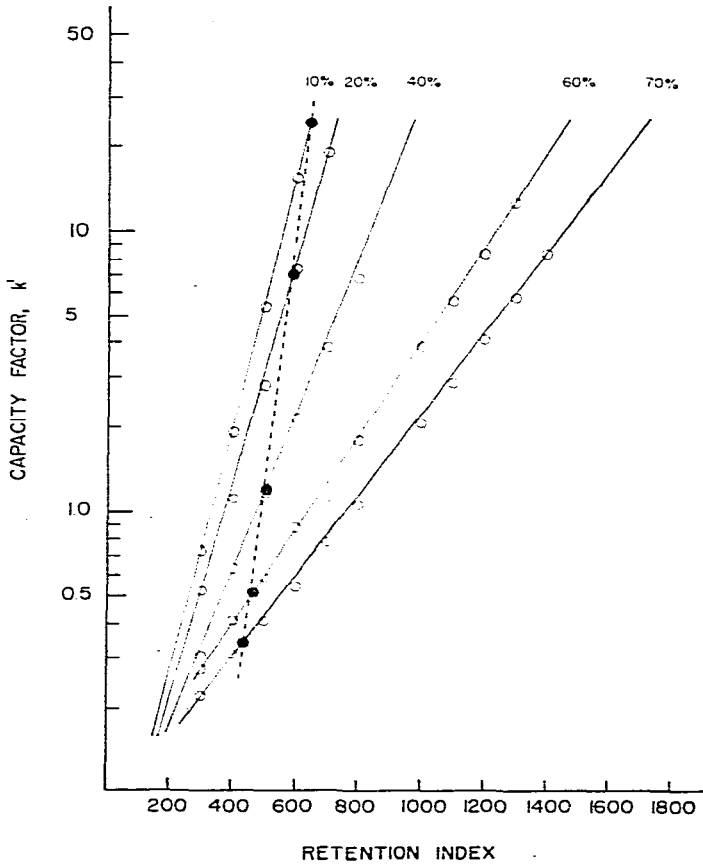

Fig. 2. Effect of solvent composition on capacity factor. ○, 2-Keto alkanes; ●, phenacetin, μBondapak C_{18} column, pH 7.0, 0.025 M NaH_2PO_4 aqueous buffer with varying acetonitrile content.

alcohol, ketone, or carboxylic acid. However, with the two chromatographic systems in the present study, the change in free energy associated with the addition of one methylene unit was reduced when methanol or acetonitrile was added to the mobile phase. As in the study by Tanaka and Thornton[5], it appeared that the change in free energy per methylene unit (as reflected in the slopes of the curves in Figs. 1–4) was approximately proportional to the water content of the mobile phase.

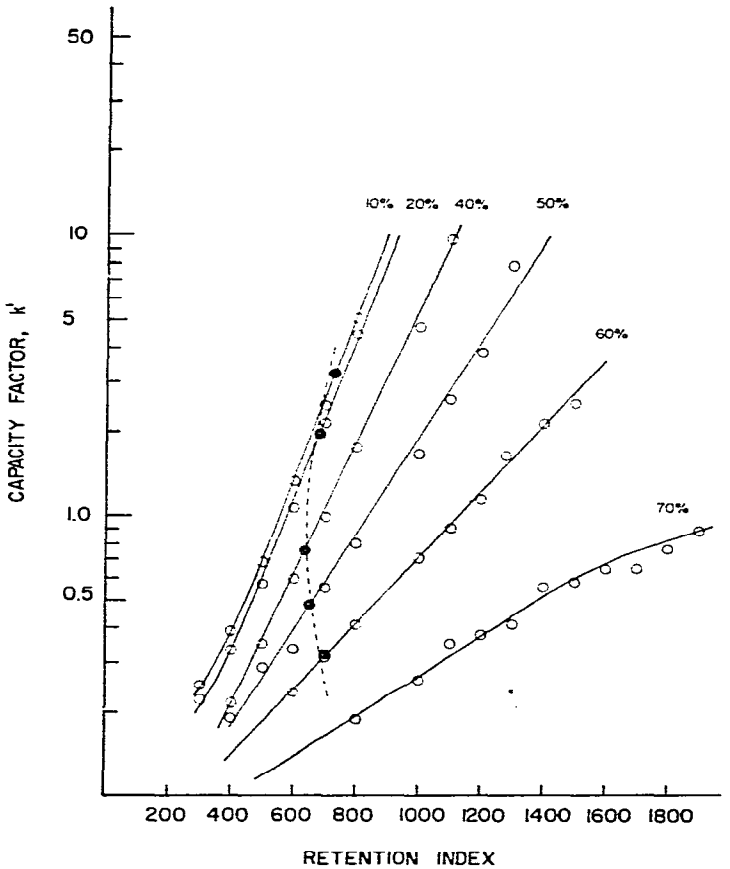

Fig. 3. Effect of solvent composition on capacity factor. ○, 2-Keto-alkanes; ●, phenacetin, μBondapak CN column, pH 7.0, 0.025 *M* NaH_2PO_4 aqueous buffer with varying methanol content.

Phenacetin was used as an example of a typical drug of moderate polarity and it was chromatographed under a wide variety of conditions with the μBondapak C_{18} column (Fig. 1). It was found that the retention index of phenacetin was fairly constant (average 530) even though the retention time of the peak varied over two orders of magnitude. In GLC analysis, the Kováts retention index of a compound will typically show a variation of 30–60 units over a 100° temperature range[2]. At first inspection then, it would seem that the HPLC retention index obtained for phenacetin was fairly constant considering the wide range of conditions, but the values showed a greater variation than with GLC analysis.

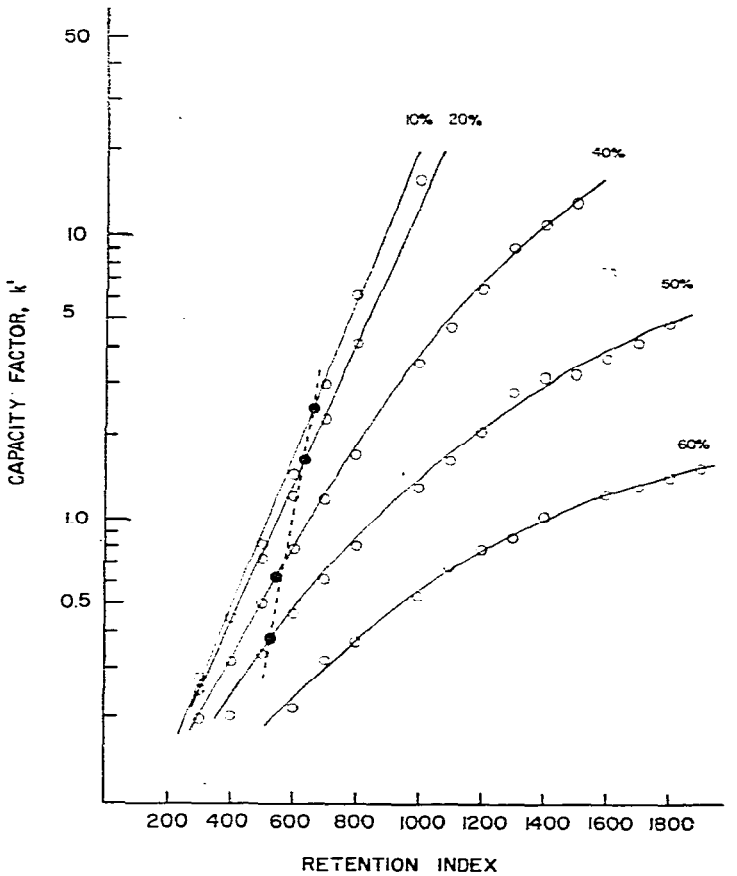

Fig. 4. Effect of solvent composition on capacity factor. ○, 2-Keto alkanes; ●, phenacetin, μBondapak CN column, pH 7.0, 0.025 *M* NaH_2PO_4 aqueous buffer with varying acetonitrile content.

The effect of solvent composition on the retention index of typical drugs representing neutral, anionic and cationic compounds was also studied. Most of the drugs were found to have slightly smaller retention indices as the percent of methanol in the mobile phase was increased (Fig. 5). Several of the compounds showed an increase in their retention indices in the 90% methanol system, however, this may be due to the reduction of the NaH_2PO_4 content from 0.025 to 0.01 *M* that was necessary to prepare this specific mobile phase (see experimental section). From the slopes of the curves in Fig. 5, it was found that the retention index of the drugs on the average decreased 18 units for each 10% increase in the methanol content of the mobile phase. Androsterone showed a fairly small decrease and the curve for acetophenone was almost flat. One could conclude that solvation effect of methanol in the mobile phase is greater for the drugs that have multiple hydrogen bonding sites than for the 2-keto alkane standards. Not surprisingly, the solvation effect on acetophenone is nearly identical to that of the standards.

The average value for the retention index of phenacetin in Fig. 1 was 530; in Fig. 2, 526; in Fig. 3, 679 and in Fig. 4, 591. In comparing these four systems, the value of k' for phenacetin was usually reduced by a half when acetonitrile replaced

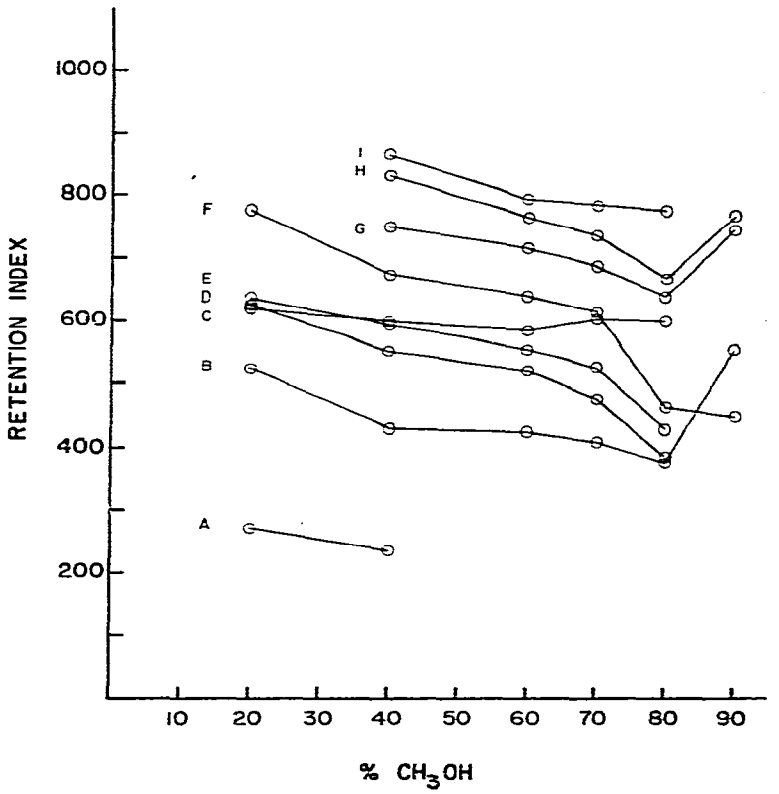

Fig. 5. Variability of the retention index of select drugs. μBondapak C_{18} column, pH 7.0, 0.025 *M* NaH_2PO_4 buffer with varying methanol content. A, aspirin; B, caffeine; C, acetophenone; D, phenobarbital; E, phenacetin; F, chlordiazepoxide; G, methaqualone; I, androsterone.

methanol in the mobile phase for the same column. This observation was consistent with the polarity indices for methanol (6.6) and acetonitrile (6.2) that have been reported[6].

The value of k' for phenacetin was considerably lower when the μBondapak CN column was used instead of the C_{18} column even when using the same mobile phase composition. However, the retention index for phenacetin was slightly higher when the μBondapak CN column was used which would indicate that the column has some selectivity for binding phenacetin but the binding forces are much weaker than with the C_{18} column.

The behavior of acetophenone with the four chromatographic systems (open circles, Fig. 6) was found to follow more closely the idealized model. All four of the curves were very flat and there were only very small differences in the retention index caused by the substitution of acetonitrile for methanol in the mobile phase. As was observed for phenacetin, the retention index of acetophenone was slightly higher with the μBondapak CN column than with the μBondapak C_{18} column.

The variation of the retention index of androsterone with the four chromatographic systems was somewhat more complex (closed circles, Fig. 6). Though the curves for the two μBondapak C_{18} systems were fairly flat, the cyano column curves

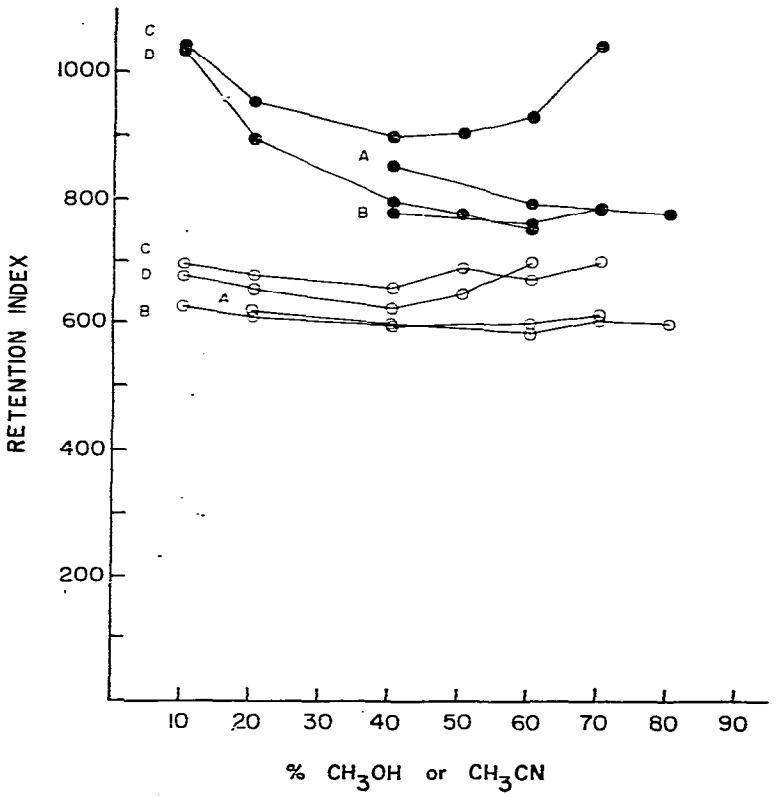

Fig. 6. Effect of column type on the retention index. ●, Androsterone; ○, acetophenone; A, μBondapak C_{18} column with methanol mobile phase modifier; B, μBondapak C_{18} with acetonitrile modifier; C, μBondapak CN column with methanol modifier; D, μBondapak CN column with acetonitrile modifier.

were concave. Though no explanation could be given for the shape of the latter two curves, the values obtained for the retention index of androsterone was slightly higher for the cyano column compared to the C_{18} column.

In the case of phenacetin, acetophenone and androsterone, it was observed that their retention indices were slightly higher when the μBondapak CN column was used if the same concentration of methanol or acetonitrile was used. Either the μBondapak CN must have some selectivity for binding phenacetin, acetophenone and androsterone or the μBondapak C_{18} column may have had a disproportionate affinity for the 2-keto alkane standards. Since the three compounds all have multiple polarizable groups or groups capable of forming hydrogen bonds, we were inclined to believe the former mechanism was true. Thus we propose that the μBondapak CN column has a slightly higher selectivity (higher retention index), but lower affinity (lower k') for the three compounds as compared to the C_{18} column. This was also true if either methanol or acetonitrile was used in the mobile phase.

In conclusion, it would appear that the retention index scale based on the 2-keto alkanes is useful in characterization of the reversed-phase chromatographic properties of drugs and other compounds. The retention index provides a continuous

scale for the reporting of chromatographic data which should be easily reproduced from laboratory to laboratory. The retention index of a given compound does not change markedly when the percent composition of the mobile phase is changed. Only slight changes in the retention index of the test compounds are observed when the type of mobile phase is changed and these changes can be related to changes in the selectivity of the system. For example, if acetic acid were added to the mobile phase, this would tend to decrease k' for most neutral compounds, decrease k' for bases and increase k' for organic acids. In terms of the retention index, the addition of acetic acid should not change the value for neutral compounds, decrease the value for bases and increase the value for organic acids.

The retention index scale is also useful for detecting changes in selectivity (as opposed to affinity) when the type of column is changed. At the present time, data is very limited, but perhaps a system such as the McReynolds constants[7] used in GLC analysis could be developed to systematize comparisons of column selectivity and mobile phase selectivity. We would suggest that for reversed-phase systems in general, a C_{18} column and pure water be used as the standard either by direct comparison or by extrapolation.

Though we found that the retention index of a given drug did not markedly change with changes in solvent composition, solvent type, or column type, it is not likely that these results can be extended to the various types of adsorption HPLC. Preliminary studies with μPorasil and μBondapak CN (adsorption mode) columns have not been very successful. For the few drugs that have been studied, the retention indices are markedly lower than obtained with any of the reversed-phase systems. As an additional problem, the 2-keto alkanes standards have too narrow a range of k' values to encompass a wide range of drugs as had been attained in the reversed-phase studies.

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