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Short communication

High-performance liquid chromatographic separation of pairs of isotopic labeled (deuterium/protium) molecules

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Abstract

The separation of *n*-octane-d_0 and *n*-octane-d_{18} has been accomplished using a liquid chromatographic technique; baseline separation was exhibited with the *n*-octane-d_{18} eluting first. Thus, the liquid chromatographic separation exhibits an inverse isotope effect just as has been observed for gas chromatographic separations. An inverse isotope effect was observed for isotopomer pairs even when they were not separated sufficiently to exhibit two distinct peaks.

Keywords: Isotopic analysis; Decane

1. Introduction

The gas chromatographic (GC) separation of isotopically labeled molecules was accomplished within a few years of the development of GC [1–12]. This technique affords a convenient method to study isotope effects. The introduction of high-efficiency GC columns in recent years opens new opportunities for the analysis of isotopic labeled molecules [13–15]. However, to the best of our knowledge, the separation of isotopically labeled and unlabeled molecules (isotopomers) using a high-performance liquid chromatographic (HPLC) method has not been reported. In this communication, we report the HPLC separation of a deuterated compound from its undeuterated counterpart.

The GC studies of the separation of pairs of isotopic molecules using DB-5 [14,15][1], SPB-5, SPB-35 and SPB-50 [16] show that the deuterated species always elutes first. This phenomenon was described as an 'inverse isotope effect' by the pioneers in this field [1–13,17–20]. The nature of this phenomenon is believed to be a vapor pressure isotope effect (VPIE). A recent review article summarized the recent developments in the area of VPIE [21]. It was of interest to learn whether such an 'inverse isotope effect' was applicable to liquid chromatographic separations.

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[1] The DB-5 column, purchased from J&W Scientific, is a 60 m×0.32 mm fused-silica column. The liquid phase was 5% diphenyl, 95% dimethylsilicone. The SPB-5, SPB-35 and SPB-50 columns are provided by Supelco. The SPB-5 and SPB-35 are 60 m×0.32 mm fused-silica columns and SPB-5 is a 30 m×0.32 mm fused-silica column. The liquid phases of these columns are: SPB-5, 5% diphenyl, 95% dimethylsilicone; SPB-35, 35% diphenyl, 65% dimethylsilicone; and SPB-50, 50% diphenyl, 50% dimethylsilicone.

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2. Results and discussion

If the 'inverse isotope effect' measured using GC was caused solely by VPIE, the same value would be obtained using different columns. However, the values obtained using the above-mentioned GC columns differ from column to column. The data in Fig. 1 show that the highest value of log V_H/V_d (a measure of the 'inverse isotope effect') for octane-d_0/octane-d_{18} is obtained using the SPB-50 column, followed in turn by the values obtained using the SPB-35 and SPB-5 columns. Interestingly, although the SPB-5 and DB-5 columns were obtained from different manufacturers, they have similar stationary phases (according to the manufacturer's literature), and the values of log V_H/V_d are essentially the same for these two columns. These results indicate that additional contributions to the 'inverse isotope effect' may result from interactions between the molecules and the stationary phase of the column. If this conclusion is valid, pairs of isotopic labeled molecules could be separated by a liquid chromatographic method.

As expected, a mixture of decane-d_{22} and decane-d_0 was separated completely by a HPLC method that utilized a C_{18} column[2] and methanol–H_2O (9:1) as the mobile phase (Fig. 2A). The first peak in Fig. 2A was identified by GC–MS to contain decane-d_{22} and the second to contain decane-d_0. Under similar conditions, octane-d_{18} and octane-d_0 were also separated even though in this instance there is some overlap between the two peaks (Fig. 2B). Again, the first peak was shown to contain octane-d_{18}. The curve in Fig. 2C shows that only one broad peak was obtained in an attempt to separate a mixture of ethylbenzene-d_{10}–ethylbenzene-d_0 under similar conditions. However, when the fractions of this peak designated as a, b and c were collected, the GC–MS data show that in fraction a, the ethylbenzene-d_{10} was enriched, and in fraction c, ethylbenzene-d_0 was enriched. Separations for methylcyclohexane-d_{14}–methylcyclohexane-d_0, ethylbenzene-ethyl-d_5–ethylbenzene-d_0, ethylbenzene-ring-d_5–ethylbenzene-d_0,

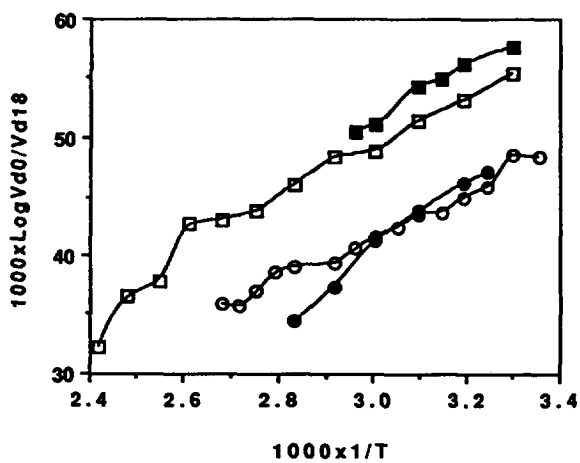

Fig. 1. Plot of the logarithms of the retention volumes of octane-d_0/octane-d_{18} vs. $1/T$ on different columns: (○) SPB-5, (●) DB-5, (□) SPB-35, (■) SPB-50.

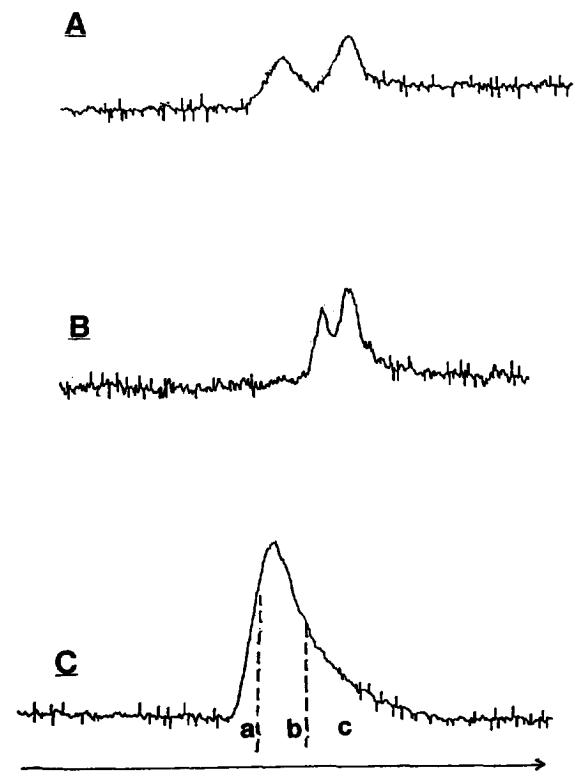

Fig. 2. HPLC chromatograms of decane-d_0–decane-d_{22} (A), octane-d_0–octane-d_{18} (B) and ethylbenzene-d_0–ethylbenzene-d_{10} (C) on an LC-18 column. Mobile phase, methanol–H_2O (9:1); flow-rate, 1.0 ml/min (A), 1.25 ml/min (B), 0.5 ml/min (C); refractive index detector.

[2] The LC-18 column was purchased from Supelco. According to the manufacturer, this column was prepared by bonding an octadecysilane reagent to the base 3 or 5 μm spherical silica.

toluene-d_8–toluene-d_0 and benzene-d_6–benzene-d_0 were attempted using the same conditions. The results obtained for these latter mixtures resemble those of ethylbenzene-d_{10}–ethylbenzene-d_0, i.e., one broad peak was obtained with the deuterium-containing material being enriched in the first portion of the peak. It seems clear that for these latter pairs, using a C_{18} column and methanol–H_2O as mobile phase, there is a discrimination in the separation process.

The advantages of HPLC separation of isotopomers are obvious and include the fact that it will be much easier to scale up than with GC as well as the fact that the compounds which are not stable under GC conditions can be separated by using a HPLC method. Most importantly, the isotopic separations by HPLC also provide an opportunity to study interactions between the molecules in the liquid phase and the stationary phase.

The difference in retention times between deuterated compound and its isotopomer is a result of differences in the intermolecular interactions between the molecules and the stationary phase. The interaction between LC-18 and decane-d_0 (f_H) is much stronger than the force between LC-18 and decane-d_{22} (f_D). It is the difference between these forces ($f_H - f_D$) that causes an additional delay for the undeuterated compound in these HPLC separations. In the theory of isotope effects, the contributions of the intermolecular and intramolecular interactions to the potential energy are usually taken to be independent of isotope effects. The kinetic energy portion of the energy depends upon mass, and therefore the isotope content. The dominant factor for the mass term is usually the impact upon the difference in the zero point energy for the initial and transition states for a chemical reaction or, in the present case, between the solution in methanol and adsorbed on the substrate. GC columns have been improved to the point where the frequencies associated with different C–H(D) bonds within a molecule such as, for example, the ring and alkyl hydrogens of ethylbenzene, may have a dramatic impact upon the retention of the compound and therefore on the magnitude of the inverse isotope effect [14]. This effect has been observed experimentally for GC [14] and it is presumed that it will apply equally in LC separations.

Since there is a discrimination in the HPLC (or LC) process towards deuterated and undeuterated molecules, special attention must be given to mechanistic studies whenever the isotope effect method is employed and HPLC (or LC) separation of isotopomers was used. Any incomplete collection of the elute may give misleading results. Investigators utilizing gas chromatography recognized that even early GC columns could operate so that there was isotope discrimination between the materials eluting during the early and later portions of a peak even though the resolution of the columns used at that time was not adequate to provide a separation of the isotopomers. Thus, if one wanted to obtain, for example, the extent of H/D exchange in a compound, it was necessary to analyze the material that made up the entire peak or to make a determination of the H/D ratio at various intervals during the evolution of the peak. The present data show that an analogous situation exists for LC. Thus, one should view with caution isotope tracer studies that utilized only a portion of the peak for isotope analyses.

At this stage, we do not know why the deuterated species always elutes first, how the molecules interact with the stationary phase of the column, and whether the solvent will affect the separation. These questions will be the subject of further study.

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