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Separating Isotopic Molecules Using SPB™ Capillary GC Columns

Improved retention times for isotopic molecules are now observed with the introduction of high efficiency capillary columns. This article describes a study comparing three SPB-series columns and their ability to perform isotopic separations quickly and accurately.

Key Words:
- isotopic molecules • aliphatic molecules
- isomers • gas chromatography

Gas chromatography (GC) is a convenient and accurate method for separating isotopically labeled (deuterium) molecules for studying the role of kinetic isotope effects in reaction mechanisms. However, GC analyses for this application typically are conducted at relatively low temperature. As a result, long retention times have been observed, limiting the use of GC in isotopic separations.

The introduction of high efficiency GC columns in recent years has improved analyses of isotopically labeled molecules (1). Conceivably, GC could be applied to economically separate larger amounts of material.

In a comparison study, we used three columns — SPB-5 (60m length), SPB-35 (60m), and SPB-50 (30m) — to determine their capability to separate isotopically labeled molecules. Table 1 shows the time and temperature needed to achieve complete, baseline separation of nine isotopic pairs. All pairs were separated completely on the SPB-5 column except ethylbenzene ring-d_5/d_0, which exhibited an overlap between the two peaks. However, all of the compounds could be determined quantitatively within the range of the experimental error (1-2%). The SPB-35 column exhibited a less effective separation of benzene-d_6/d_0 and toluene-methyl-d_3/d_0. The other pairs, however, had larger separation factors because of the relatively longer retention times.

Table 1. Conditions Needed for Complete Separation of Isotopically Labeled Molecule Pairs

	SPB-5		SPB-35		SPB-50	
Isotopic Pair	**Temp. (°C)**	**Time (min)**	**Temp. (°C)**	**Time (min)**	**Temp. (°C)**	**Time (min)**
Benzene-d_6/d_0	25	6	30	14*	**	**
Toluene-methyl-d_3/d_0	30	11	50	16*	**	**
Toluene-d_8/d_0	60	7	70	9	30	4*
Methylcyclohexane-d_{14}/d_0	60	6	60	7	40	2
Octane-d_{18}/d_0	80	6	90	5	30	3
Ethylbenzene-ring-d_5/d_0	35	23*	50	33*	**	**
Ethylbenzene-ethyl-d_5/d_0	60	10	80	12	30	9*
Ethylbenzene-d_{10}/d_0	80	7	80	12	50	4
Naphthalene-d_8/d_0	110	12	140	17	80	16

*An overlap exists between the two peaks.

**The two compounds were not separated.

Data provided by Center for Applied Energy Research, University of Kentucky, 3572 Iron Works Pike, Lexington, KY 40511 USA.

Since the SPB-50 column used in the study is a shorter length, comparison with the other two columns is difficult. However, methylcyclohexane-d_{14}/d_0, octane-d_{18}/d_0, and ethylbenzene-d_{10}/d_0 were separated completely in four minutes or less.

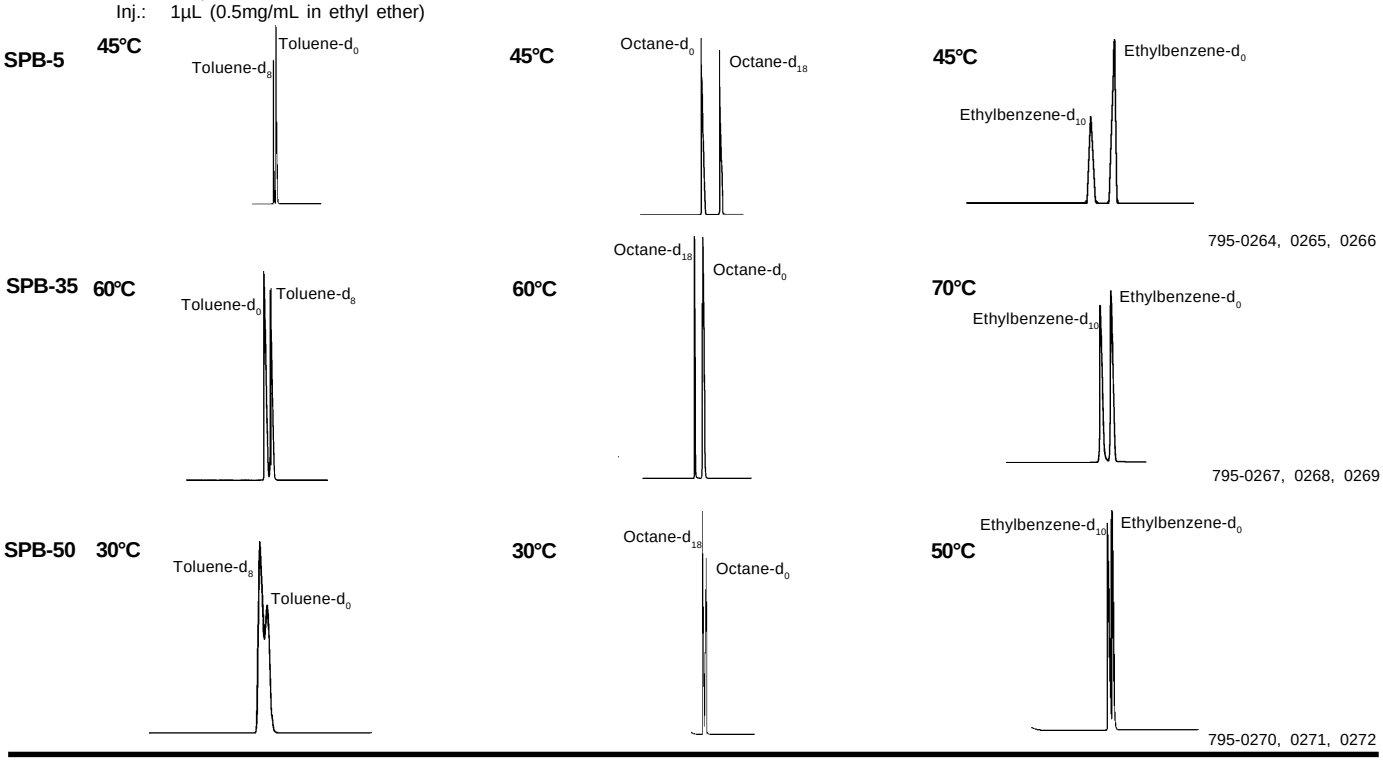
Representative chromatograms of toluene-d_5/d_0, octane-d_{18}/d_0, and ethylbenzene-d_{10}/d_0 separations using all three SPB columns are shown in Figure A. The heavier (more deuterated) isomer always elutes first in a phenomenon known as the inverse isotope effect. The vapor pressure isotope effect is the major contributor to the inverse isotope effect on GC.

In studying the mechanism for the dehydrocyclization of alkanes using a Pt/SiO_2 catalyst, we used octane-d_{18}/d_0 and/or methylcyclohexane-d_{14}/d_0 as the feed to define the extent of deuterium/hydrogen exchange and the kinetic isotope effects (2). In this reaction, the major products are benzene, toluene, o-xylene, and ethylbenzene. Unreacted starting reagents and other compounds are also contained in the reactor effluent. The amount of each component, including deuterated components, have been determined quantitatively by using GC methods. Figure B shows a standard mixture of possible components from these reactions obtained on an SPB-5 column. From our experience, the GC method gives a more accurate analysis of the deuterium contents than some other methods, such as deuterium NMR and infrared.

For a mixture containing both aliphatic and aromatic pairs of isotopic molecules, the SPB-5 column appears to offer the best separation. For a mixture containing only aliphatic molecules, SPB-35 and SPB-50 columns are the best choice because of reduced elution time.

Figure A. Comparative Separation of Isomers on Three Capillary Columns

Column: **SPB-5, 60m x 0.25mm ID, 0.25µm film**
SPB-35, 60m x 0.25mm ID, 0.25µm film
SPB-50, 30m x 0.25mm ID, 0.25µm film
Carrier: helium
Det.: FID, 280°C
Inj.: 1µL (0.5mg/mL in ethyl ether)

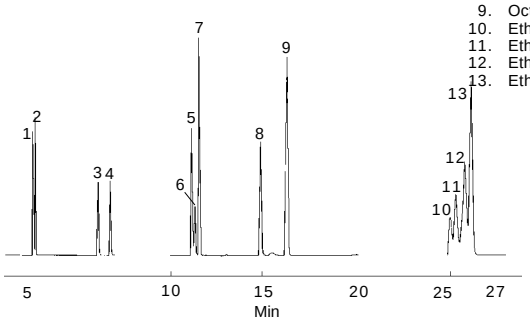


Chromatograms provided by Center for Applied Energy Research, University of Kentucky, 3572 Iron Works Pike, Lexington, KY 40511 USA.

Figure B. Standard Mixture of Dehydrocyclization of Alkanes

Column: **SPB-5, 60m x 0.25mm ID, 0.25µm film**
Cat. No. **24036**
Oven: 25°C (5 min) to 45°C at 4°C/min
Carrier: helium
Det.: FID, 280°C
Inj.: 1µL (0.5mg/mL in ethyl ether)

1. Benzene-d_6
2. Benzene-d_0
3. Methylcyclohexane-d_{14}
4. Methylcyclohexane-d_0
5. Toluene-d_8
6. Toluene-methyl-d_3
7. Toluene-d_0
8. Octane-d_{18}
9. Octane-d_0
10. Ethylbenzene-d_{10}
11. Ethylbenzene-ethyl-d_5
12. Ethylbenzene-ring-d_5
13. Ethylbenzene-d_0



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Chromatogram provided by Buchang Shi, Center for Applied Energy Research, University of Kentucky, 3572 Iron Works Pike, Lexington, KY 40511 USA.

Ordering Information:

Fused Silica Capillary Columns

SPB-5, 60m x 0.25mm ID, 0.25µm film	**24036**
SPB-35, 60m x 0.25mm ID, 0.25µm film	**24093**
SPB-50, 30m x 0.25mm ID, 0.25µm film	**24181**

References

1. Shi, B. and H. Davis, *J. Chromatogr., 654,* 319 (1993).
2. Shi, B. and H. Davis, *J. Catal., 147,* 38 (1994).

Fused silica columns manufactured under HP US Pat. No. 4,293,415.

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