The dead time is recalculated based on volume and flow rate and inserted in 2.2 of methodology. From: http://www.chromatographyonline.com/column-dead-time-diagnostic-tool

The dead time is calculated from the following equation:

$$t_d = \frac{\text{Volume}}{\text{Flow rate}}$$

For this study, $t_d = 1.5 \text{ mL}/1.0 \text{ mL/min} = 1.5 \text{ minutes}$

Calculated dead time is 1.5 min. Retention time of B6 is 1.6 min. If we add extra-column contribution (capillary from injector to column and capillary from column to detector), dead time is about 1.6 min. The conclusion is that vitamin B6 is not retained. Our chromatographic method is thus unusable for separation of vitamin B6 in any mixture.
<table>
<thead>
<tr>
<th>HPLC Troubleshooting Guide</th>
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<tr>
<td>The injector rapidly introduces the sample into the system with minimal disruption of the solvent flow.</td>
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</table>

**FROM:**

With a sufficiently small injection volume, we can assume that all the sample molecules arrive at the head of the column at the same time, and as they migrate down the column, the peak spreads into the familiar Gaussian shape.