New HPLC columns, or columns which have been previously used for different chromatographic analyses must be conditioned, or equilibrated, with the desired mobile phase before a sample may be injected. Run the solvent at 1 mL/min for 30–60 min. This is equivalent to 10 to 20 column volumes of mobile phase for the standard 4.6mm id x 250mm analytical or WVS column.

The time actually needed for equilibration varies with the media, the mobile phase to be used, and the mobile phase used in the past. For example, reversed phase columns equilibrate fully within 10 minutes at a flow rate of 1ml/min of methanol/water or acetonitrile/water mobile phases. Changing the pH of a Partisil™/Partisphere™ SAX or SCX, on the other hand, could require one-half to two hours to reach column equilibrium, while ionic strength changes can be accomplished in 10 to 20 minutes.

When changing from one mobile phase to another, each mobile phase must be miscible in the other. When the two mobile phases do not readily mix, an intermediate solvent is required. For example, when changing from heptane to methanol, an intermediate solvent such as ethyl acetate is required.

Preparative columns contain more packing than their analytical counterparts. It is necessary, therefore, to run correspondingly greater volumes of solvent through the column to achieve complete equilibration.

A simple way to determine when the column is in equilibrium is to set the detector at high sensitivity and wait for the appearance of a stable baseline. Where UV detectors are used, the baseline may stabilize before complete equilibration. In these cases, inject a standard and measure the retention time; when duplicated retention times are obtained on two sequential runs, the column can be said to be equilibrated.

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