

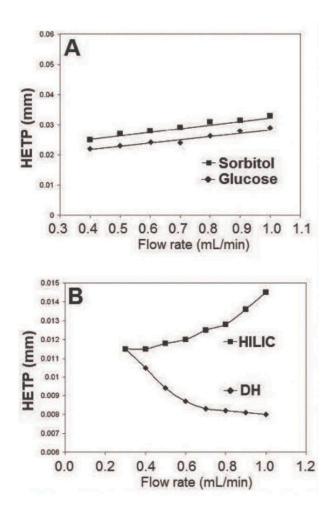
Efficiency of ANP Measured - AppNote

Aqueous Normal Phase (ANP) Efficiency Measured with Sorbitol, Glucose & Uracil

Figure A is an excerpt from a study that investigated analyses of Glucose and Sorbitol on TYPE-C Silica based HPLC Columns and found that the efficiency of ANP is better than observed with HILIC. A Cogent Diamond Hydride Column (2.1 x 150mm with a Particle Size of 4 μ m) was used and Flow Rate v. HETP was plotted as shown. From the plot it is evident that while the Flow Rate is increased by a factor of 2.5 the Efficiency is only slightly lower (~15%).

A similar Cogent Diamond Hydride ($4.6 \times 150 \text{mm}$ and Particle Size of $4 \mu \text{m}$) and a typical, commercially available HPLC Column designed for HILIC mode, (with equal specifications) were used to retain and separate Uracil under the same conditions. Figure B shows a plot of "Efficiency vs. Flow Rate" for both Columns. From the graph it can be seen that efficiency increases as the Flow Rate increases from $0.3 \times 1.0 \text{mL}$ / minute for the ANP Column while in contrast, the HILIC Column's efficiency dropped by more than 30% over this same range of Flow Rates.

It should be noted that linear Flow Rates are more than 6 times greater in the 2.1mm Column than in the 4.6mm.



Figures:

A. Sorbitol, Glucose. On a Diamond Hydride Column B. Uracil. DH and HILIC Column Comparison

Method Conditions

Columns:

Figure A: Cogent Diamond Hydride™, 4µm, 100Å Figure B: Cogent Diamond Hydride™, 4µm, 100Å

Catalog Nos.:

Figure A: <u>70000-15P-2</u> Figure B: <u>70000-15P</u>

Dimensions:

Figure A: 2.1 x 150mm Figure B: 4.6 x 150mm

Mobile Phase:

A: DI Water / 0.1% Formic Acid B: Acetonitrile / 0.1% Formic Acid Flow rate: Variable, See Charts

Detection: UV (*Uracil*) and LCMS (*Sorbitol*, *Glucose*)

Injection vol.: 10µL

Notes: Charts and Methods are adapted from "Analysis of Hydrophilic Metabolites in Physiological Fluids by HPLC-MS using a Silica Hydride-Based Stationary Phase". J.J. Pesek, M.T. Matyska, J.A. Loo, S.M. Fischer, T.R. Sana, J. Sep. Sci., 32



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