# Comparison of Denaturing and Nondenaturing Mobile Phases in SEC: ZORBAX GF-250, +/- SDS

## Application

## Biochemical

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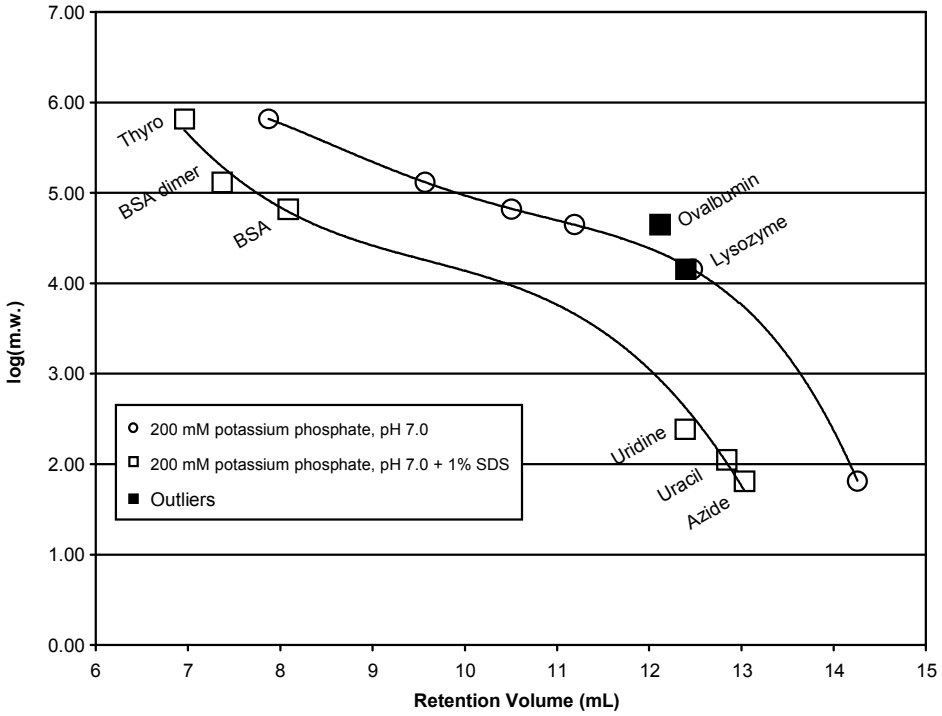
Size-exclusion chromatography is a powerful technique for the size-dependent separation of biomolecules. While standard, non-denaturing mobile phases are often desirable, denaturing mobile phases containing guanidine hydrochloride (or SDS), may be used to insure un-aggregated, monomers for proper size determination. As shown below, denaturation shifts molecules toward larger apparent size and smaller retention volume.




**Conditions:**
**LC: Hewlett-Packard 1050**
**Column: ZORBAX GF-250 (9.4 x 250), Agilent Part No. 884973-901**
**Det.:UV: 254 nm**
**Flow: 2.0 mL / min.; ambient**
**Inj. Vol.: 20 µL (1 µg / µL)**

### *Highlights*

- *Agilent ZORBAX GF-250 columns separate biomolecules in a size-dependent manner within a molecular-weight range of 4,000-400,000 daltons. Note, some molecules (depending on their characteristics) are retained by non-SEC mechanisms and are shifted from linearity.*
- *ZORBAX GF-250 columns are manufactured using extremely hard particles, allowing high flow rates and fast separations.*
- *When larger molecules are used in combination with denaturing mobile phases, ZORBAX GF-450 may be used to obtain more-linear separation in the upper molecular-weight range.*

**Agilent Technologies**

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