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## ACCEPTED MANUSCRIPT

#### **Retention and Effective Diffusion of Model Metabolites on Porous Graphitic Carbon**

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#### Highlights

- Hypercarb shows increased retention of metabolites compared to a C18 bonded phase
- Retention on Hypercarb allows for focusing of large injections in gradient elution
- Potential impact of effective diffusion on gradient runs studied using peak parking
- Diffusion characteristics make Hypercarb suitable for long gradient runs

#### Abstract

The study of metabolites in biological samples is of high interest for a wide range of biological and pharmaceutical applications. Reversed phase liquid chromatography is a common technique used for the separation of metabolites, but it provides little retention for polar metabolites. An alternative to C18 bonded phases, porous graphitic carbon has the ability to provide significant retention for both non-polar and polar analytes. The goal of this work is to study the retention and effective diffusion properties of porous graphitic carbon, to see if it is suitable for the wide injection bands and long run times associated with long, packed capillary-scale separations. The retention of a set of standard metabolites was studied for both stationary phases over a wide range of mobile phase conditions. This data showed that porous graphitic carbon benefits from significantly increased retention (often > 100 fold) under initial gradient conditions for these metabolites, suggesting much improved ability to focus a wide injection band at the column inlet. The effective diffusion properties of these columns were studied using peak-parking experiments with the standard metabolites under a wide range of retention conditions. Under the high retention conditions, which can be associated with retention after injection loading for gradient separations,

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