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# Evaluation of a C18 hybrid stationary phase using high-temperature chromatography

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## Abstract

Alkyl benzenes and aromatic alcohols were used as model analytes in a chromatographic separation on a new type of hybrid C18 stationary phase. The stationary phase was characterized from the point of view of its interaction with the mentioned analytes. The thermodynamic parameters such as $\Delta H^\circ$ and $\Delta S^\circ$ showed that there are very few differences in the interactions of the alkyl benzenes and aromatic alcohols with the stationary phase in the temperature interval from 150 to 200 °C. A temperature study in the range from 30 to 200 °C using toluene as an analyte revealed that the van't Hoff plot is non-linear. Differential scanning calorimetry studies on this stationary phase indicated a phase transition at ~90 °C. Studies on the influence of organic modifier concentrations indicated that the retention of the analytes at 150 °C varied linearly with the volume fraction of acetonitrile in the mobile phase. At 150 °C the van Deemter plot showed that a minimum was reached for the reduced plate height at ~1.6 mL/min which was constant up to 5 mL/min. A 1-month stability study of the column using pure water as a mobile phase, at 200 °C indicated that analyte retention factor changed by 10% during this period of time.

*Keywords:* Alkyl benzenes; Aromatic alcohols; High-temperature chromatography; Thermodynamics; van Deemter plot; Hybrid stationary phase

## 1. Introduction

Temperature is one of the most important parameters that can influence the selectivity of HPLC separations, as well as the resolution between particular components in a mixture. There are just a few examples of chromatography that can be cited that use high temperature as a means of enhancing separation. Size exclusion chromatography of polymers with a low solubility [1], technical waxes [2], etc., are often performed at temperatures ranging between 140 and 150 °C in halogenated aromatic solvents; similarly, ion exchange chromatography is used at high temperatures to improve the exchange capacity [3]. One of the reasons that temperature is not used frequently in HPLC separations is the lack of stationary phases that are stable at high temperatures [4] even though recently, several papers and reviews have appeared outlining the use of stationary phases in conjunction with high-temperature chromatography [5–7]. Furthermore, temperature can decrease the partition coefficient and, as a consequence, reduce the retention of a particular analyte. As a matter of fact, temperature can decrease analyte retention up to 5%/°C [8]. In addition, the viscosity of the mobile phase decreases at elevated temperatures, allowing for faster flow rates and shorter analysis times [9]. There are also impediments when working at high temperatures. For example, temperature mismatch between the mobile phase and the column has to be considered. Such mismatches can cause band broadening that becomes significant at higher flow rates [10]. Another impediment of high-temperature chromatography is the possible thermal decomposition of the analytes. Therefore, the time a compound spends in a particular chromatographic column should be minimized [4].

The use of pure high-temperature water (HTW) as a mobile phase at temperatures above 100 °C brings a new dimension to chromatographic separations. The solvation properties of

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pressurized hot water changes compared to ambient liquid water [11]. At the same time, changes in the degree of analyte ionization can be significant at high temperatures [12]. HTW is structurally different from ambient liquid water. Hydrogen bonding, which gives water its unique properties, becomes weaker as the temperature rises. At the same time the increase in temperature produces a decrease in density. Thus, unlike the infinite percolating network of hydrogen bonds found in ambient liquid water, the hydrogen bond network in HTW exists in the form of small clusters of hydrogen-bonded water molecules [13]. With increasing temperature and decreasing density, the average cluster size decreases. Changes in the extent of hydrogen bonding are accompanied by corresponding changes of the dielectric constant. As temperature increases, the dielectric constant of water decreases and HTW behaves more like polar organic solvents. Consequently, small organic compounds become soluble in HTW. The breaking of the hydrogen bond network also reduces the barrier for translational and rotational motion of individual water molecules. This effect contributes to the increase in the self-diffusivity of water with increasing temperature and decreasing density [13]. Additionally, HTW offers other advantages such as being a very good medium for organic chemistry [13] and an environmentally friendly solvent. For example, many geochemical reactions, such as the conversion of kerogen into petroleum, occur in high-temperature water [14].

The column packing material used for this study belongs to the family of C18 hybrid organic-inorganic packings [15]. Such packings are prepared by the copolymerization of tetraethoxysilane as the precursor of the inorganic component, with an organofunctional silane as the organic component. With proper design, the resulting particles inherit the advantageous properties of high-purity silica-based particles, such as their mechanical strength, combined with the advantageous properties of polymeric particles, such as their pH stability. The specific attributes of such packing materials are a function of the selection of the organic component. The first commercial inorganic–organic hybrid packing material based on this technology was synthesized with methyltriethoxysilane as the organic component [15]. This initial packing material already possessed significant improvements in pH stability over the classical silica-based packing materials [16]. The incorporation of a large amount of methyl groups (or other organofunctional groups) reduces the amount of silanol groups on the surface of the packing. As a consequence, the achievable surface coverage in a bonding reaction is reduced. For the first generation methyl hybrid, the surface coverage is limited to 2.5 $\mu$mol/m$^2$. The remainder of the surface is covered with methyl groups, and consequently, very little residual silanol activity is observed with these packing materials [17]. One reason for the low-silanol activity is the low-silanol content [17]. The other reason is a shift in the p$K_a$ of the silanols towards the alkaline pH [18]. The majority of the silanols on fully hydroxylated high-purity silica have a p$K_a$ value around 7, while the p$K_a$ of the methyl hybrid packing, measured with the same techniques, shifts to a value of around 10 [18,19].

Table 1
Physical characterization of the hybrid stationary phase

| Particle | Bridged hybrid |
|---|---|
| Particle %C (%) | 5.6 |
| Surface area (m$^2$/g) | 189 |
| Pore diameter (nm) | 14.8 |
| Pore volume (mL/g) | 0.76 |
| Total %C (%) | 15.9 |
| Surface coverage ($\mu$mol/m$^2$) | 3.0 |

The improved pH stability of methyl hybrid packings results in a wide array of new options in their use for chromatographic separations. The expanded pH range allows an even better exploitation of pH as a tool in method development [20,21]. A careful examination of the different options available in reversed-phase chromatography used to manipulate the selectivity of a chromatographic separation shows that the change of pH results in the largest selectivity differences for ionizable compounds, superior to using a packing material with another surface or a different organic modifier in the mobile phase. In addition, the preparative loadability improves by a factor of about 20 or more when the compound to be separated is converted from an ionic form to a non-ionic form [22]. Thus, a basic pH permits the preparative separation of basic compounds in the non-ionic form using a methyl-hybrid packing.

The incorporation of methyl groups into a packing material is only one of many options. Longer chains and chains with functional groups are also possible. Both options significantly expand the array of properties that can be used to design new packings. Recently, ethyl-bridged hybrid packings were designed by the co-condensation of l,2-bis(triethoxoxysilyl)ethane with tetraethoxysilane [23]. Studies show a further improvement in the pH stability of the ethyl-bridged hybrid packings over the first generation methyl hybrid. The improved pH stability also promises that the temperature stability of the ethyl-bridged hybrid under reversed-phase condition should be much improved. The properties of the experimental ethyl-bridged hybrid packings used in the present study are shown in Table 1.

In the present paper, we report the separation of alkyl benzenes and aromatic alcohols with the aim of characterizing the behavior of a hybrid C18 stationary phase using HTW and combination of HTW and acetonitrile modifier as mobile phases.

## 2. Experimental

### 2.1. Chemicals and reagents

HPLC grade acetonitrile was purchased from EM Science (Gibbstown, NJ, USA). HPLC grade water was generated by a Milli-Q water system (Millipore, Bedford, MA, USA). Uracil (>99.0% purity), toluene (99.8% purity), ethylbenzene (99.0% purity), propylbenzne (98.0% purity), butylbenzene (>99.0% purity), amylbenzene (99.0% purity), isobutylbenzene (99.0% purity), *tert*-butylbenzene (99.0% purity), *sec*-butylbenzene (99.0% purity), benzyl alcohol (>99.0% purity), 4-phenyl-l-butanol (>99.0% purity), phenylethyl alcohol (99.0% purity), 3-phenyl-l-propanol (98.0% purity), and 5-phenyl-pentanol (99.0% purity) were purchased from Sigma–Aldrich (St. Louis, MO, USA).