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Comparison of the elution behavior of aliphatic, aromatic, and heteroatomic compound classes on four different normal-phase HPLC columns

Bryan Verbist, Aleksandra Gorska, Damien Pierret, Giorgia Purcaro

Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium

Abstract

The growing demand for efficient and selective separation of complex mixtures in both industrial and academic contexts underscores the need for a better understanding of compound class behavior in liquid chromatography. Generally, the optimal choice of stationary phase for separating specific classes of compounds is unclear, particularly when dealing with chemically diverse analytes. To address this challenge, this study evaluates and compares the elution and separation performance of a broad range of analytes using four distinct UHPLC stationary phases: Allure™ Silica (bare silica), Ultra® Amino (amino-bonded silica), Nucleosil® Chiral-2 (donor-acceptor complex chromatography, DACC), and Inertsil® Diol (diol-bonded silica). The Megamix standard mixture, consisting of 76 compounds from diverse aliphatic, aromatic, and heteroatomic classes, was used for the analysis. These compounds exhibit a wide range of polarities, aromatic characteristics, and functional groups. Elution profiles on the different columns were assessed to identify trends in retention behavior, selectivity, and class-based resolution. The Allure™ Silica column demonstrated strong selectivity for polar compounds, effectively clustering them by chemical composition, but showed prolonged retention of highly polar analytes, leading to longer run times. The Nucleosil® Chiral-2 column offered excellent separation of aromatic compounds according to their number of aromatic rings, largely due to strong electron donor-acceptor (EDA) interactions linked to HOMO energy. However, this column showed reduced performance for non-aromatic compounds and partial overlap between classes, influenced by alkylation-related retention. The Inertsil® Diol column exhibited moderate polarity and retained oxygenated and chloro-oxygenated compounds effectively. It enabled fine intra-class separation among polar compounds and displayed the highest repeatability of all tested columns. In contrast, the Ultra® Amino column, with higher polarity than Diol and improved chemical stability over bare silica, provided good separation of oxygenated compounds from others but lacked selectivity for less polar compounds and had lower repeatability than Diol.