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# Combined effects of mobile phase composition and temperature on the retention of phenolic antioxidants on an octylsilica polydentate column

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ABSTRACT

Combined effects of temperature and mobile-phase composition on retention and separation selectivity of phenolic acids and flavonoid compounds were studied in liquid chromatography on a polydentate Blaze $C_8$ silica based column. The temperature effects on the retention can be described by van't Hoff equation. Good linearity of $\ln k$ versus $1/T$ graphs indicates that the retention is controlled by a single mechanism in the mobile phase and temperature range studied. Enthalpic and entropic contributions to the retention were calculated from the regression lines. Generally, enthalpic contributions control the retention at lower temperatures and in mobile phases with lower concentrations of methanol in water. Semi-empirical retention models describe the simultaneous effects of temperature and the volume fraction of the organic solvent in the mobile phase. Using the linear free energy-retention model, selective dipolarity/polarizability, hydrogen-bond donor, hydrogen-bond acceptor and molecular size contributions to retention were estimated at various mobile phase compositions and temperatures. In addition to mobile phase gradients, temperature programming can be used to reduce separation times.

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## 1. Introduction

Polyphenols are a large family of natural compounds widely distributed in plants, usually as esters or glycosides conjugated with alcohols, hydroxy- or fatty acids, sterols, etc. Many of them possess antioxidant properties, such as protective role against cancer and coronary heart diseases [1,2]. The beneficial effects of this class of compounds on human health have fostered continuously increasing interest in the analysis of natural antioxidants in food, beverages and plants.

Polyphenolic compounds are usually conveniently analyzed using reversed-phase (RP) HPLC. However, separations of polyphenolic acids and many other strongly polar natural antioxidants require highly aqueous RP systems and these compounds can be often better separated by non-aqueous normal-phase (HILIC) chromatography [3]. On the other hand, some flavonoids – especially aglycons – are rather non-polar and mobile phases with a higher concentration of organic modifier are necessary to accomplish their elution. For strongly retained compounds, high-temperature HPLC provides shorter separation times and often improves separation selectivity and efficiency [4–9].

The theory of high-temperature chromatography has been developed earlier [4–9]. Later, it has been extended to describe simultaneous effects of solvent composition, pH and temperature on the retention of ionizable compounds [10–12]. The linear solvent strength model was used as the basis of the theory of temperature gradients [13] and models were developed which allow prediction of retention times when simultaneously changing solvent composition and temperature [14,15]. Other models were introduced to calculate the retention in two-mode gradient lution with simultaneously changing temperature and mobile phase composition [16]. Nikitas and Pappa-Louisi overviewed one- and multi-variable retention models for isocratic and gradient elution models, involving variations in mobile phase composition, pH and temperature [17].

Unfortunately, high temperature operation and applications of programmed temperature in HPLC are still relatively rarely used in practice, because of the lack of suitable stationary phases and instrumentation enabling adequate control of fast changes in column temperature. Some silica-based stationary phases commonly used in contemporary HPLC practice are not stable at temperatures higher than 60 °C. Therefore, high-temperature HPLC has become subject of systematic research only recently, when stationary phases with improved temperature stability at elevated temperature have become available [18,19]. Many of them are based on matrices other than silica, such as polystyrene/divinylbenzene adsorbents or other organic polymers, graphitized carbon or metal

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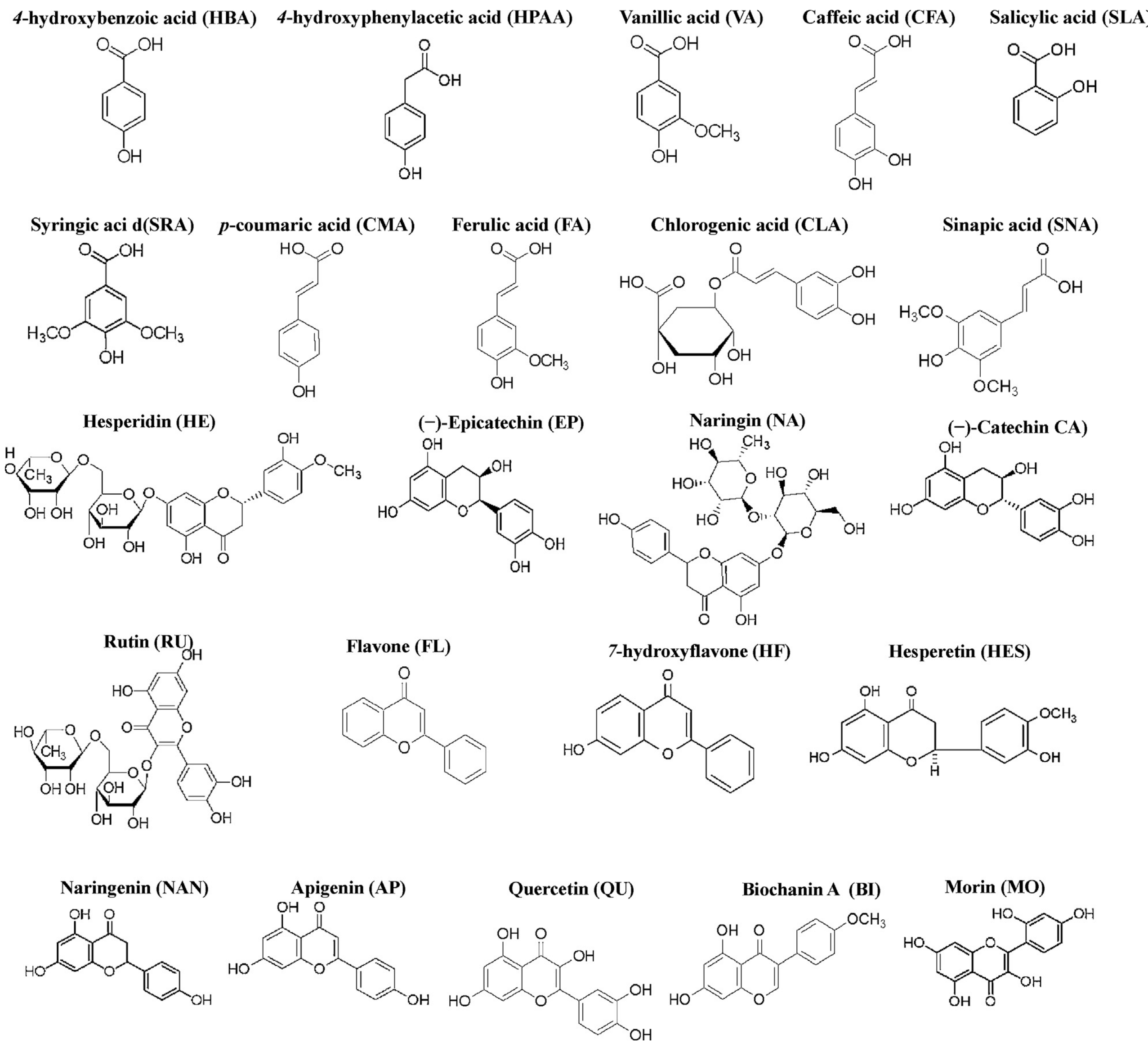


**Fig. 1.** Structures of phenolic acids and flavonoid antioxidants.

oxide stationary phases [20], based mainly on zirconium oxide with deposited polymer or carbon layers, or with chemically bonded alkyl chains [20,21]. These materials show excellent thermal stability and can be used at temperatures higher than 100 °C [20]. Unfortunately, they show lower efficiency than the silica based column packings [21] and often too strong retention for some compounds such as flavonoids, resulting in excessively long elution times and/or strongly tailing peaks even at temperatures higher than 100 °C [22].

For these reasons, new technologies were sought to prepare thermally stable silica-based materials. Thermal stability of silica gel materials can be enhanced via hydrosilation process, in which up to 95% of silanols (Si—OH) on the surface of silica are replaced by almost non-polar silane (Si—H) groups [23,24]. Chemically bonded hydrosilated silica stationary phases, are stable up to 80–100 °C [25]. Recently, we investigated the mobile phase and temperature effects on the chromatographic behavior of phenolic acids [26] and flavones [27] on hydrosilated silica type columns (Cogent Hydride, Cogent Diamond, Cogent UDC cholesterol and Cogent bidentate C18). Such thermally stable stationary phases allowed running temperature gradients up to 180 °C on Zirconia polybutadiene columns and on hybrid Waters Acquity Phenyl and Waters XBridge C18 columns [28,29].

Silica-based polydentate reversed phase materials with multiple-site bonded group attachment to the silica gel surface show high efficiency and can be used at temperatures over 100 °C. Good thermal stability is attributed to the shielding effect of the polydentate bonded ligands, which protect the surface of the silica gel against hydrolysis at increased temperature [30].

Recently, we investigated combined effects of mobile phase composition and temperature on the retention of a variety of homologous and polar compounds on a thermally stable polydentate bonded silica-based column, Selerity Blaze $C_8$ [22]. In the present work, we extended this study to gradient elution and programmed temperature conditions. We focused the attention on the separation of natural phenolic and flavone antioxidants on this column, taking into account the sample structural effects. Phenolic acids of interest contain – besides of an carboxylic group – one to three phenolic —OH and one or two methoxy groups; flavonoids contain 0–5 —OH groups, methoxy group(s) and one or two sugar moieties (Fig. 1). As the earlier employed benzene derivative model standards with one or two more or less polar substituents [30]

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