Study on the application of chemically modified synthetic copolymer columns for liquid chromatographic food analysis

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区　　分　：甲

論　文　内　容　の　要　旨

Chromatographic separation of analytes in matrices is essential for qualitative and/or quantitative evaluation of food quality. Liquid chromatographic (LC) separation techniques are a convenient analytical tool in not only food science field, but also other scientific fields, and are growing by combinatorial use of mass-spectrometry (MS). Aiming at adequate LC separation of polar compounds, hydrophilic interaction chromatography (HILIC) column is commonly used for exploing their hydrophilic interaction. Hydrophobic compounds can be separated on reversed-phase column by hydrophobic interaction. However, successful LC separation of a given analyte may result in column separation capacities such as hydrophilic and hydrophobic interaction. Silica-based resins for conventional LC column may be limited for diverse chemical modifications. Thus, a complete separation of analytes seems to be difficult in a single LC column packed with silica-based resins, and required for several separation steps. In this study, copolymer-based LC resins of glycidyl methacrylate-ethylene glycol dimethacrylate and ethylstyrene-divinylbenzene, both of which are capable for designing chemical modification, were, therefore, applied for food analysis in a single LC-run.

Firstly, rare sugars (D-allose, D-psicose, D-sorbose, and D-tagatose), showing bioactivity by low-calorie characteristics, were targeted for a single LC separation by refractive index-HPLC, since their similar molecular properties such as size and polarity caused poor separation on a common HILIC column. In order to enhance the retention of reducing aldoses on column, polyethyleneimine moiety (8 wt%) with strong base property was induced into a copolymer glycidyl methacrylate-ethylene glycol dimethacrylate unit. Under the elution condition of 90 v/v% acetonitrile at a flow rate of 0.9 mL/min only non-reducing ketoses (D-psicose, D-tagatose, D-fructose, and D-sorbose) were detected and separated on the modified HILIC column (4.6 mm I.D × 150 mm, 5 μm). In contrast, reducing aldoses (D-glucose, D-allose, and D-xylose) were not eluted from the column by strong ionic interaction between aldose and imine moieties. Thus, aiming to reduce the ionic interaction on the HILIC column or to elute aldoses from the column, ion-pair reagents were applied as additive in mobile phase. As a result of optimized HPLC elution conditions, the addition of 5 mmol/L sodium 1-octanesulfonate to 85 v/v% acetonitrile (pH 4.8) allowed a complete separation of both aldoses and ketoses in a single HILIC-run within 40 min. Taken together, a single polyethyleneimine-induced glycidyl methacrylate-ethylene glycol dimethacrylate copolymer HILIC column can be extensively applied for either discriminant analysis of non-reducing monosaccharides or simultaneous analysis of non-reducing and reducing monosaccharides in different mobile phase modes.

Secondly, an ethylstyrene-divinylbenzene copolymer column (2.1 mm I.D × 100 mm, 4 μm) was applied for the separation of hydrophobic targets. In order to establish a single LC separation of analytes showing similar molecular properties such as hydrophobicity and size, catecholamines and their metabolites

were targeted in this study in combination to MS detection with high molecular selectivity by mass unit. As a result of negative-mode LC-MS analysis using a gradient elution of 0–50 v/v% methanol containing 0.1 v/v% formic acid (FA) at 0.20 mL/min, analytes bearing amino groups (dopamine, norepinephrine, and epinephrine) failed the selective MS detection by their poor separation on the copolymer column, while 3,4-dihydroxy phenylacetic acid, homovanillic acid, and 3-methoxy-4-hydroxyphenylglycol bearing no amino groups were successfully detected by their hydrophobic interaction with the column. To overcome the poor separation of catecholamines with amino groups, sulfonyl group (0.81 wt%) was induced into the copolymer unit, providing cation-exchange capacity. Under the optimized LC-MS conditions with a linear gradient elution of water containing 0.1 v/v% FA to 50 v/v% acetonitrile in 50 mmol/L ammonium formate at 0.20 mL/min, all the analytes were separated and detected within 25 min. This indicates that the copolymer column capable for chemical modification with ion-exchange groups allows a simultaneous separation of analytes showing diverse ionic and hydrophobic properties in a single chromatographic run. In the established LC-MS using the mixed-mode sulfonated ethylstyrene-divinylbenzene copolymer column, dopamine, norepinephrine, and homovanillic acid were successfully detected in mouse brain at >4 pmol/mg.

In conclusion, the present study demonstrated that a single LC column packed with copolymer resins that are capable for chemical modification with designated groups could allow for extensive analysis of targets in complex matrices without several separation steps.