Characteristics of Diol column and retention behavior of hydrophilic compounds in Hydrophilic Interaction Chromatography (HILIC)

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1. Introduction

Hydrophilic Interaction Chromatography (HILIC) is a variation of normal phase chromatography with the distinction that the mobile phase consists of organic solvent (e.g. acetonitrile) in water or an aqueous buffer. HILIC columns consist of bare silica or a polar phase (diol, amino, zwitterion, etc.). HILIC can retain and separate strongly polar compounds that are not retained in reversed phase chromatography, and can gain enhanced sensitivity in mass spectrometry. Popular HILIC columns tend to have inferior reproducibility and durability. This study investigated the retention behavior of polar compounds, reproducibility, and durability in silica gel bonded with dihydroxypropyl column YMC-Pack Diol-NP.

2. Characteristics of Diol column

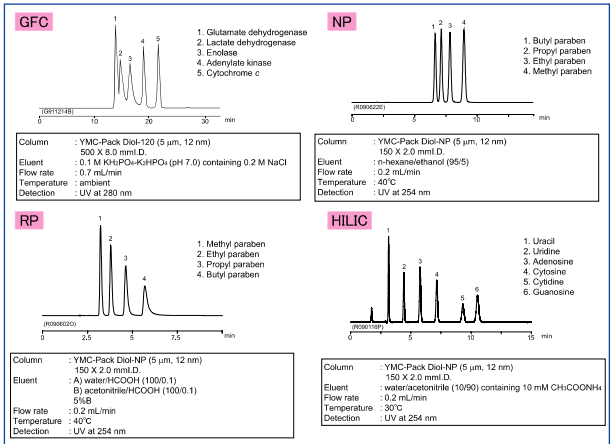

Fig. 1 Analyses of four chromatography modes in the diol column.

A diol column is used with several modes (gel filtration (GFC), normal phase (NP), reversed phase (RP), and HILIC) in chromatography (Fig. 1). They are used mainly as GFC using the mobile phase of aqueous buffer and NP with the mobile phase of organic solvent. In addition, they are used as HILIC and RP using water or an organic solvent, in HILIC, the mobile phase contains a high concentration of organic solvent. In contrast, in RP, it contains a low concentration of the organic solvent.

3. Retention behavior of hydrophilic compounds

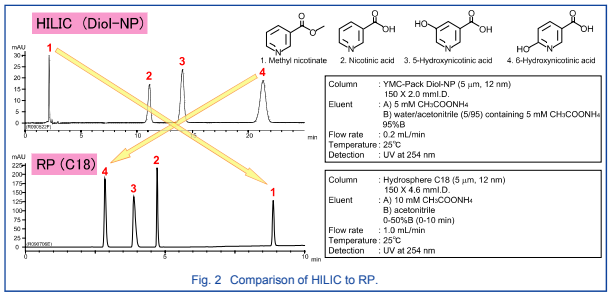

Fig. 2 Comparison of HILIC to RP.

The respective elution orders of polar compounds of HILIC and RP (nicotinic acid, methyl nicotinate, 5-hydroxynicotinic acid, 6-hydroxynicotinic acid) were compared (Fig. 2). Hydroxynicotinic acid, a polar compound, is retained strongly in HILIC. In RP, methyl nicotinate, a non-polar compound, is retained. The elution order of HILIC is opposite that of RP.

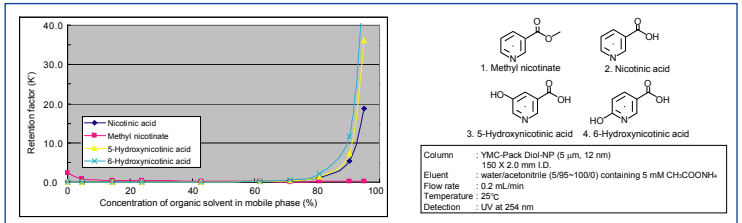

Fig. 3 Effect of acetonitrile concentration.

The amount of organic solvent in the mobile phase strongly affects the retention of polar compounds (e.g. nicotinic acid and related compounds; Fig. 3). The high organic solvent ratio (>70%) in mobile phase increases the retention of polar analytes as a result of HILIC behavior. Only non-polar analytes, methyl nicotinate, exhibit reversed-phase behavior at a low concentration of accetonitrile (<10%). The separation behavior depends on the water/acetonitrile ratio in mobile phase in the diol column.

4. Analysis of melamine and related compounds

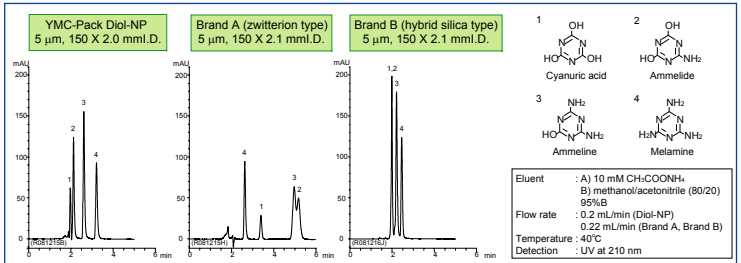

Fig. 4 Analyses of melamine and related compounds.

The separation of melamine and related compounds is compared among Diol-NP and other conventional HILIC phases (hybrid silica, zwitterion type; Fig. 4). Only Diol-NP achieves baseline separation of these compounds. Diol-NP provides favorable retention and resolution.

5. Analyses of melamine-spiked powdered cheese

I. Comparison of melamine-spiked powdered cheese on Diol-NP and RP columns

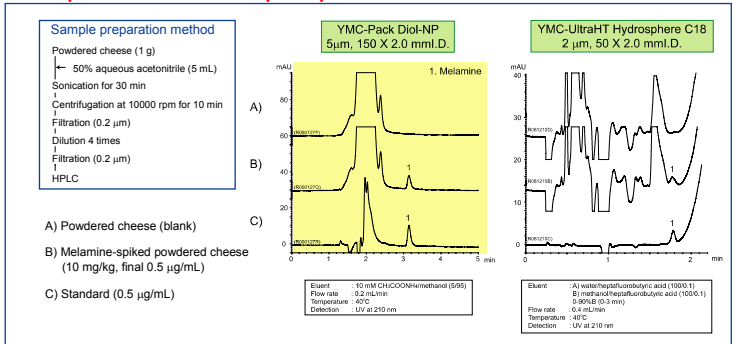

Fig. 5 Comparison of melamine-spiked powdered cheese on Diol-NP and RP column.

The separation characteristics of extract obtained from melamine-spiked powdered cheese in HILIC and RP are compared (Fig. 5). In RP, the ODS column cannot achieve favorable resolution, but in HILIC, Diol-NP shows superior resolution in analyses involving numerous matrix compounds.

II. Comparison among Diol-NP and other HILIC phases

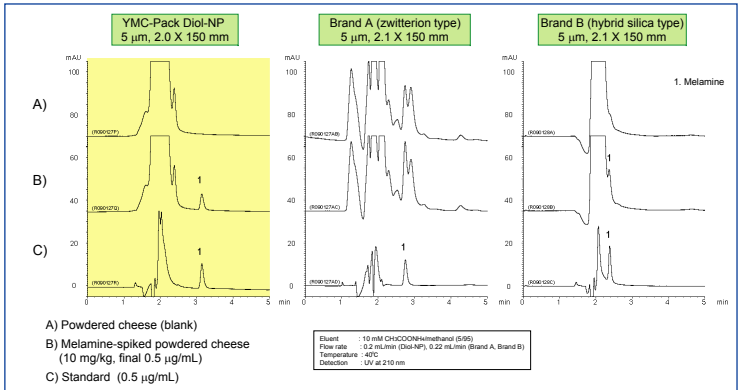

Fig. 6 Comparison among Diol-NP and other HILIC phases.

Diol-NP was compared with other HILIC phases in terms of separation of melamine-spiked powdered cheese (Fig. 6). The analyses showed that Diol-NP provides the longest retention and best resolution among all columns. Zwitterion type column selectivity is different from those of diol and silica columns. Diol-NP provides long retention and achieves baseline separation because it has neutral functional groups and adequate hydrophobicity.

III. Reproducibility and repeatability

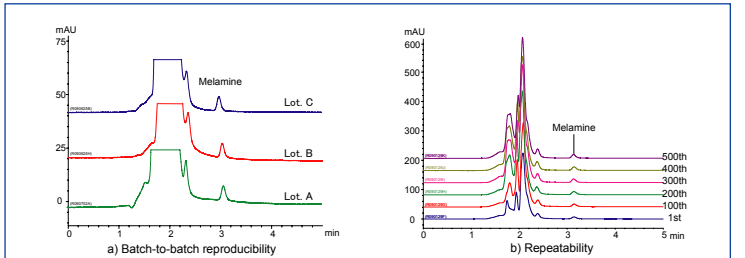

Fig. 7 Reproducibility and repeatability in Diol-NP.

HILIC columns tend to have worse reproducibility and durability than RP columns because of surface characteristics of materials and limits of the washing solvent. In Diol-NP, reproducibility of the three lots is good (Fig. 7a). Furthermore, the repeatability test chromatograms of every 100th run show no change in retention or peak shape; Diol-NP shows exceptional durability (Fig. 7b) because Diol-NP has fewer nonspecific adsorptions. Bare silica, zwitterion type, and amino type columns interact with ion exchange groups and adsorb ionic compounds. For that reason, these columns must change the selectivity and repeat washing by water. Consequently, Diol-NP shows excellent durability and repeatability.

6. Conclusions

- In Diol-NP, polar compounds are retained strongly, the elution order is opposite that of RP.
- In the analysis of melamine-spiked powdered cheese, Diol-NP provides longer retention and better resolution than zwitterion type and hybrid silica columns.
- Diol-NP shows excellent batch-to-batch reproducibility.
- Diol-NP shows no change in retention or peak shape after the 500th analysis involving many matrix compounds.