Abstract

Hydrophilic Interaction Liquid Chromatography (HILIC) proposed by Aport in 1990 has been applied for analysis of hydrophilic compounds. Amide, diol, polyal, bare silica, ion exchange and twinner ion phases have been used as a hydrophilic stationary phase along with an organic-solvent rich mobile phase. A polar group embedded C18 or a long alkyl chain phase such as C30 or C28 also have been used to separate hydrophilic compounds without change in retention using an aqueous mobile phase on a reversed phase. This is because pressure gradient caused by capillary is less atmospheric pressure, so that retention doesn’t change. Both HILIC stationary phases and reversed-phases have completely opposite characteristics each other. Therefore both HILIC and reversed-phase modes are useful for separation of hydrophilic compounds. It is important to understand separation behavior of each phase. In this study, an amide column and a C28 column were compared and evaluated to separate hydrophilic compounds. SunHill HILIC-Amide and SunHill RP-AQUA (C28) and SunHill RP-AQUA (C28) (C28) were used to separate nucleobases, amino acids and hydrophilic vitamins. When nucleobases were separated on HILIC reversed-phase modes using an amide column and a C28 column, each elution order of samples is said to be opposite. Only uracil, however, showed a specific elution. It was considered that the polarity of uracil under an organic-solvent rich condition is different from that on water rich condition due to keto-enol tautomerization. LC/MS analysis of amino acids was achieved using C28 column and a mobile phase added 5 mM acetic acid under gradient elution conditions. &Nucleobases were separated using an amide column and a C28 column, each elution order of samples is said to be opposite.

Amide vs C28

Comparing between amide and C28 column

- Each elution order of samples is almost opposite.
- Only elution order of uracil did not change.

Figure 1. Comparison between reversed phase and HILIC

Amide vs other HILIC phase

Comparing between SunHill HILIC-Amide and other HILIC columns

- SunHill HILIC-Amide showed stronger retention than others.
- Amide showed different selectivity comparing with bare silica HILIC column.

Figure 2. Comparison of three kinds of HILIC column

Applications of HILIC

Other brand aqua type C18 column.

1. Sunniest RP-AQUA, Ralf Scott
2. Jutvik, Norikazu Nagae
3. Acentice

Table 1. Physical property of each compounds

Test condition

- C28 column can be used under the 100% aqueous mobile phase condition.
- C28 column is useful column for separation of hydrophilic compounds.
- C28 column has better stability to acidic and basic conditions than a polar group embedded C16 column.

Stability of C28 under acidic and basic conditions

Test condition

- C28 column has better stability.
- C28 column was subjected to 100% aqueous mobile phase condition.
- C28 column is useful for separation of hydrophilic compounds.

Stability of C28 under acidic and basic conditions

- C28 column can be used under the 100% aqueous mobile phase condition.
- C28 column is useful column for separation of hydrophilic compounds.
- C28 column has better stability to acidic and basic conditions than a polar group embedded C16 column.

Separation of amino acids with C28

- C28 column has better stability.
- C28 column has better stability.
- C28 column has better stability.

Applications of C28

- C28 column is useful column for separation of hydrophilic compounds.
- C28 column has better stability to acidic and basic conditions than a polar group embedded C16 column.

Conclusions

- Nucleobases were separated using an amide column and a C28 column, each elution order of samples is said to be opposite.
- Only uracil showed a specific elution. It was considered that the polarity of uracil under an organic-solvent rich condition is different from that on water rich condition to be due to keto-enol tautomerization.
- LC/MS analysis of amino acids was achieved using C28 column and a mobile phase added 5 mM acetic acid under gradient elution conditions.
- Both amide and C28 column were useful for analysis of hydrophilic compounds.