C8 columns for metabolite analysis!!

CAPCELL PAK C8 DD

Introduction of metabolite analysis application and comparison of separation characteristics with ODS columns

Separation patterns unavailable from the C18 group

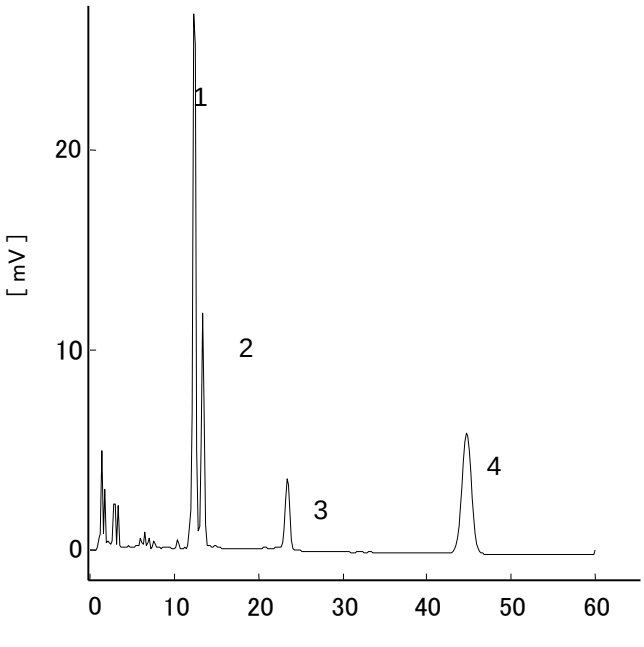
Separation patterns specific to C8 yet with high durability equivalent to C18!

C8 group-introduced columns have the following advantages:
- ►High density of introduction of the functional group because of small molecular volume of the alkyl silane agent to be introduced
- ►Good balance between polarity and hydrophobicity of the surface of packing materials because the alkyl chain is short

Best for analyzing the mixture of polar compounds having a skeleton similar to that of highly hydrophobic compounds, including drug substances and their metabolites.

●Example of analyzing antibiotics and their metabolites

Cyclosporine is a polypeptide consisting of 11 amino acids gained from the culture solution of Tolypocladium inflatum Gams. Because of its strong immunological effect, cyclosporine has been widely used since 1978 as a drug that prevents organ rejection after transplantation of organs such as kidneys. However, disposition of cyclosporine has significant intra-individual and inter-individual variability. Because the therapeutic concentration range is limited, therapeutic drug monitoring (TDM) is indispensable for gaining adequate immunosuppressive effect without grave side effects including kidney and liver damage. Shown below is an example of analyzing cyclosporine and its metabolites by the use of standard goods.



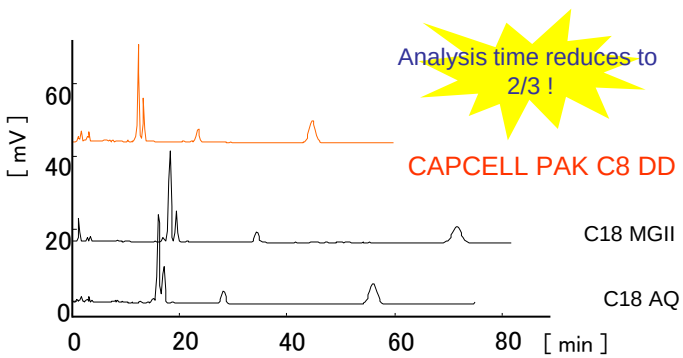
1. Metabolite AM1
2. Metabolite AM9
3. Metabolite AM4N
4. Cyclosporine A

* Note that these three metabolites (1 to 3) are highly polar compounds for the bulk pharmaceutical chemicals (4) to be easily metabolized in vivo and excreted from the body.

[HPLC Conditions]
Column: CAPCELL PAK C_8 DD S5 ; 2.0 mm i.d. x 150 mm
Mobile phase: 55vol% CH_3CN
Flow rate: 200 µL/min
Temperature: 70 ℃
Detection: UV 210 nm
Injection vol.: 4µL
Sample: Mixed solution of aliquot product dissolved in CH_3OH

C8 DD reduces 1/3 of analysis time -Comparison of separation patterns between C8 and C18 columns-

Shown below is a comparison with C18 columns: C18 MGII with large retention and C18 AQ with high surface polarity.



This comparison reveals that C8 DD is almost equivalent to C18 MGII in the separation of peaks of highly polar metabolites. Moreover, C8 DD reduces about 1/3 of the retention time of cyclosporine, the most highly hydrophobic drug substance. In this example, even C18 AQ (with the surface of packing materials having the highest polarity) did not show good separation of peaks of metabolite with small retention. This may be attributed to the hydrophobicity of the compounds.
These results reveal that the C8 column with excellent separation characteristics delivers a good performance in metabolite separation.

●Good retention and separation of polar compounds and fast elution of highly hydrophobic compounds

The following are the key points in analyzing sampled mixture of polar compounds and hydrophobic compounds in reversed-phase partition mode:

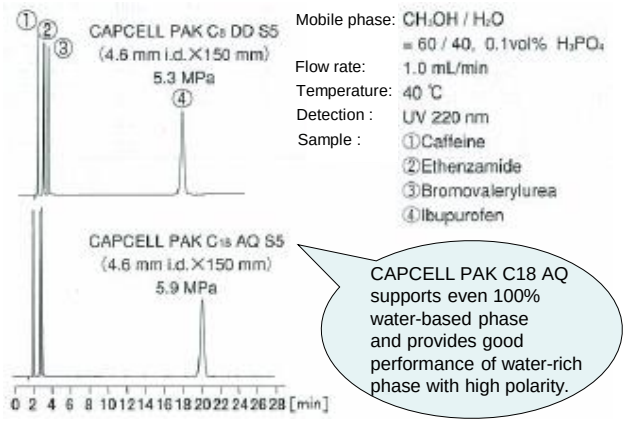
・Polar compounds provide good retention and separation.
・Hydrophobic compounds have not so great retention and reduce analysis time.

CAPELL PAK C8 DD has high polarity of the surface of packing materials, because the introduced alkyl chain has lower hydrophobicity than the C18 group, while the density of introduction of the functional group where interaction takes place is high.

These features provide excellent performance in analyzing sampled mixture of polar compounds and hydrophobic compounds.

◆Analysis of cold medicine ingredients◆

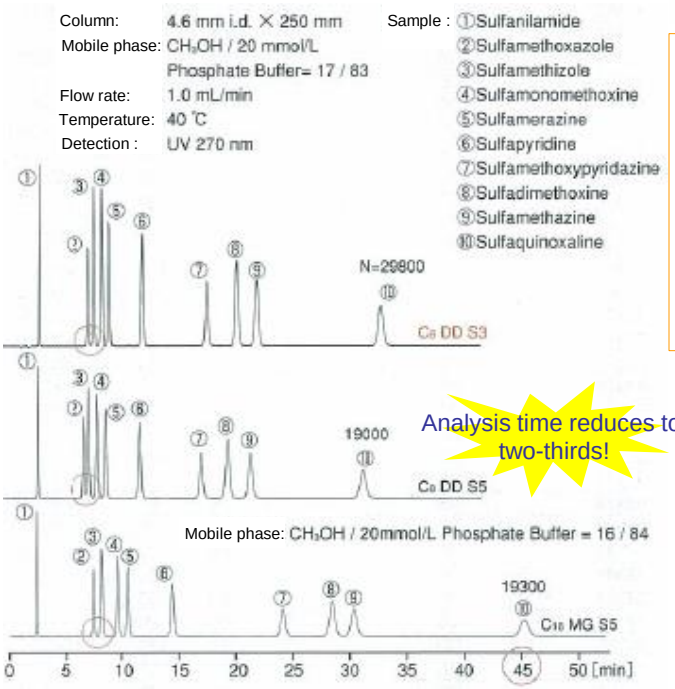
CAPCELL PAK C8 DD provides greater retention and separation of the peaks of ethenzamide (2) and bromovalerylurea (3) with high polarity. It also provides faster elution of ibupurofen, which has rather high hydrophobicity, than C18 AQ characterized by smaller retention than other C18 columns.



◆Analysis of sulfonamide◆

In comparison with C18 columns, CAPCELL PAK C8 DD eluted highly hydrophobic compounds in a shorter time under the conditions of retention and separation of a group of compounds with small retention and high polarity.

A column with smaller particle size would further improve separation in other component analysis.



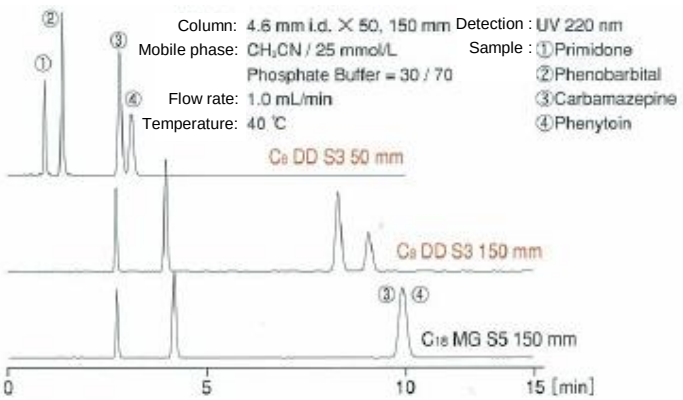
●Separation pattern specific to C8

In reversed-phase partition mode, separation is made based on the partition difference between stationary and mobile phases of the target component. So, separation differs between C8 and C18 columns because of the good balance of packing materials of C8 between hydrophobicity.

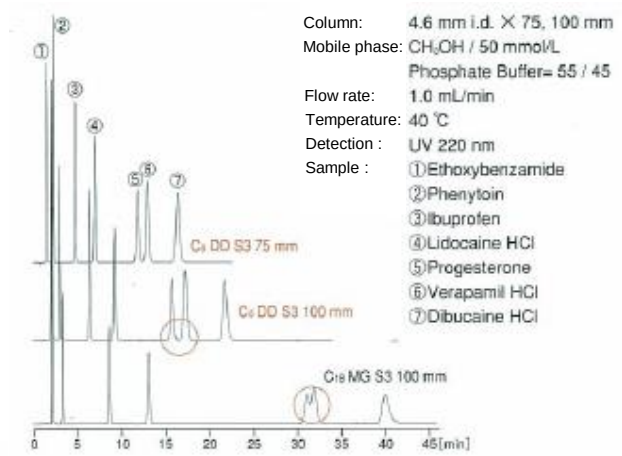
Shown below are examples of analyzing an antiepileptic drug and a cold medicine. Both of them reveal the difference of separation behavior between C18 and C8 columns under the same conditions. CPCAELL PAK C8 DD shows shorter time of overall analysis and good separation.

A 3-µm short column would have further reduced the analysis time.

◆Analysis of antiepileptic drug◆



◆Analysis of cold medicine◆



●Features of C8 retention behavior:
Generally shorter analysis time in comparison with C18 columns
Separation patterns different from those with C18 columns

●Try it for such analysis as the following:
Multi-component analysis of the mixture of polar compounds and hydrophobic compounds
Coincidence measurement of bulk pharmaceutical chemicals and their metabolites
Analysis of components with the same basic skeleton (such as antibiotics)

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