CAPCELL PAK ADME, the novel reversed stationary phase modified by adamantyl functional group

Abstract

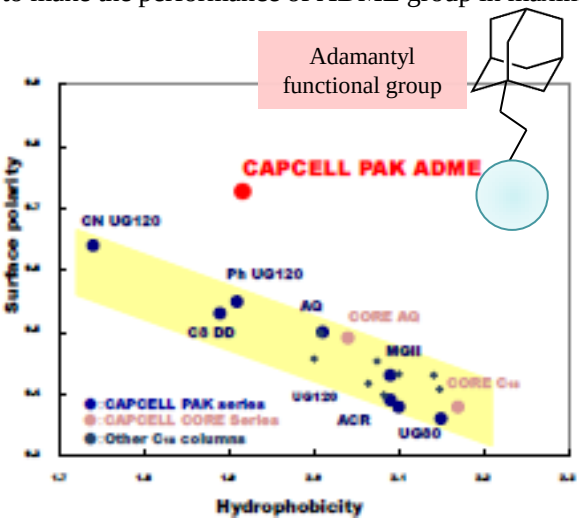
C_{18} is the most popular stationary phase used in reversed-phase chromatography, but has difficulty in retain highly polar compounds. Capcell Pak ADME (ADME) is developed by firstly introducing adamantyl groups as the functional group of the reversed stationary phase of a HPLC column. The changed alkyl structure of adamantyle group provides ADME separation characteristics with a balance between moderate hydrophobicity and extra-high surface polarity which is completely different from the conventional C_{18}. In this study, the characteristics of ADME and its applications compared with C_{18} columns in the analysis of metabolite are reported.

Key words: Adamantyl functional Group, polar compound, metabolite, HPLC, LC-MS.

Experiment & Results

Characteristics of ADME

The hydrophobicity and Surface Polarity of Capcell Pak ADME were measured with 10 standard-reagents method (S. Kobayashi etc. *J. Chromatogr. A*, **1998**, 828, 75-81) and plotted in the parameter graph among Capcell Pak series, Capcell Core series and other conventional C_{18} columns (Fig.1). Capcell Pak ADME positionsat extra-high surface polarity and moderate hydrophobicity whichis unavailable with any other polar columns and conventional C_{18} columns. Based on the Capcell Pak technology,the implementation rate is tightly controlled at an optimized density in order to make the performance of ADME group in maximum.



[Fig. 1a] Parameter of hydrophobicity and polarity

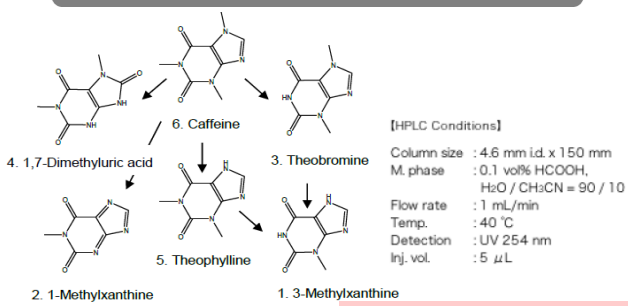
Physical characteristics of ADME (Table 1)

Particle Size (μm)	Micro pore Diameter (μmm)	Special Surface (m^2/g)	Density (μmol/m^2)	C %	pH range
5	10	300	2.8	12	2-9
3	10	300	2.8	12	2-9

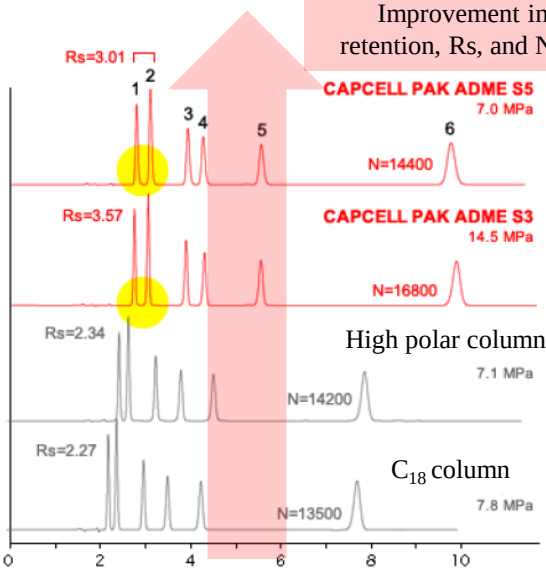
Capcell Pak ADME Vs C18 columns

With the novel balance between hydrophobicity and polarity never experienced by other reverse-phase columns, ADME provide improved separation to high polar compounds such as the metabolites. In the simultaneous analysis of of metabolites of caffeine (Fig.2) and tryptophan(Fig.3), ADME provided improved separation compared with the conventional high polar columns and C_{18} columns.

Improved separation with ADME (isocratic)



Improvement in retention, Rs, and NTP

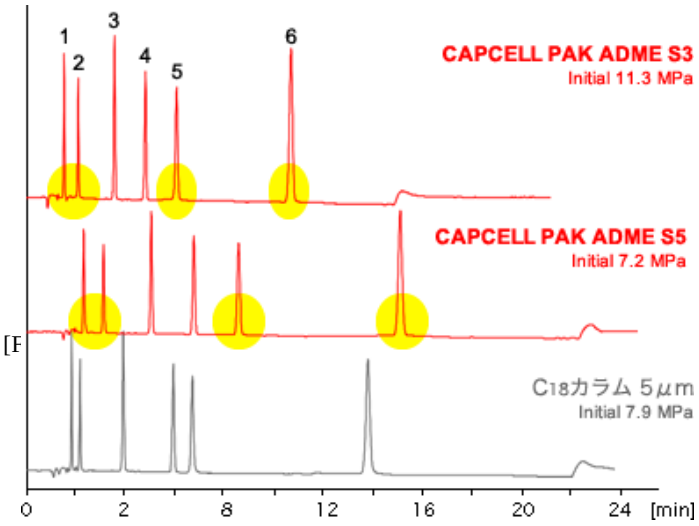


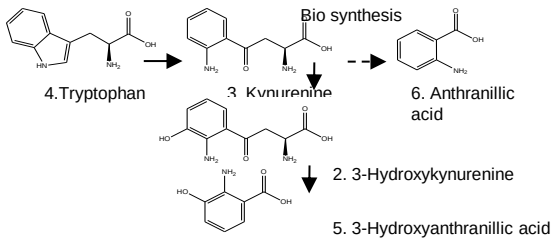
[Fig. 2] Chromatograms of Xanthenes

Capcell Pak ADME Vs C_{18} columns

Capcell Pak ADME strongly retains the highly polar compounds in the process of metabolism of the tryptophan (Fig 3a) due to its large surface polarity, and provides improved separation even to the compounds hard to retain by the conventional C_{18} columns. Especially, ADME could advance the retention of high polar compounds much more under a gradient condition. ADME provided excellent separation capacity to retain the highly polar metabolites of tryptophan with a wide analysis window. In addition, downsizing of particle size to 3μm further improved the number of theory plate (NTP) and resolution.(Fig. 3b).

Improved separation of metabolites (gradient)





[Fig. 3a] Metabolism pathway of tryptophan

[HPLC Conditions]
Column size : [S3] 4.6 mm i.d. x 100 mm, [S5] 4.6 mm i.d. x 150 mm
Mobile phase : A) 10 mmol/L Ammonium formate buffer (pH4)
B) CH_3CN
[S3] B 5% (0 min) -> 25% (13.3 min) -> 5% (13.4 min) Gradient
[S5] B 5% (0 min) -> 25% (20.0 min) -> 5% (20.1 min) Gradient
Flow rate : 1 mL/min
Temperature : 40 °C
Detection : UV 254 nm
Inj. vol. : 1 μL
Sample : 1. Uracil, 2. 3-Hydroxykynurenine, 3. Kynurenine, 4. Tryptophan, 5. 3-Hydroxyanthranillic acid, 6. Anthranillic acid

[Fig. 3b] Chromatograms of tryptophan and its metabolites

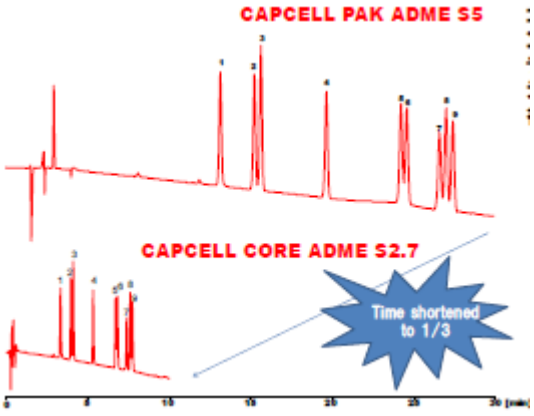
Lineup of Capcell Core ADME

9 steoid hormone in the Cholesterol biosynthetic pathway were certainly separated with Capcell Pak ADME (5μm) and its core-shell type. Fast simultaneous analysis was reached simply by switching ADME to Capcell Core ADME (2.7μm) with arrows 2-fold linear velocity and short gradient program. (Fig. 4)

【HPLC Conditions】
Column : ①CAPCELL PAK ADME S5 ; 4.6 mm i.d. x 150 mm
: ②CAPCELL CORE ADME S2.7 ; 2.1 mm i.d. x 50 mm
Mobile phase : A) 5 mmol/L $HCOONH_4$ B) CH_3CN
① B 20 %(0 min) -> 55 %(30 min) -> 20 % (30.1 min) Gradient
② B 20 %(0 min) -> 55 %(10 min) -> 20 % (10.1 min) Gradient
Flow rate : ① 1 mL/min, ② 400 μL/min
Temperature : 40 °C; Detection : UV 220 nm
Inj. vol. : ① 5 μL, ② 25 μL
Sample : 1. Aldosterone, 2. Cortisol, 3.Cortisone, 4. Corticosterone, 5. 17β-Estradiol, 6. Testosterone, 7. Estrone, 8. Ethynylestradiol, 9. 17α-Hydroxyprogesterone

[Fig. 4] Chromatograms of tryptophan and its metabolites

Fast analysis of 9 metabolites with Capcell Core ADME



Conclusion & Discussion

Capcell Pak ADME and Capcell Core ADME firstly introduced the adamantyl group as the functional group of reverse-phase columns and provide improved separation of highly-polar compounds. ADME is expected as an alternative to C_{18} column in simultaneous analysis of samples having diverse polarities such as metabolites and fast separation of polar compounds.

Website & contact

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