2018/07/28–08/02
HPLC2018
P-T-1309

Poly(4-vinylpyridine) Based Novel Stationary Phase Investigated under Supercritical Fluid Chromatography

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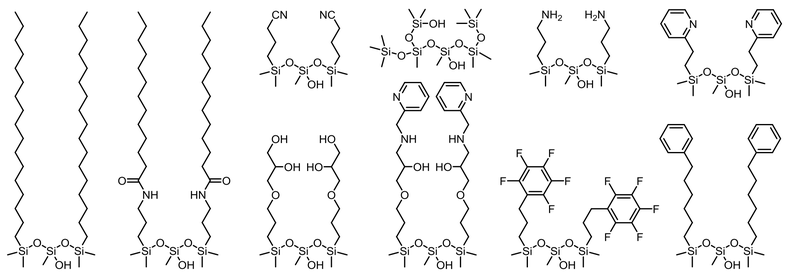
1. Introduction

Commercially Available Achiral Stationary Phase

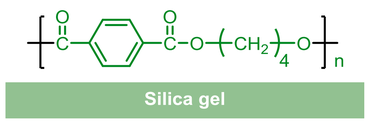


Most achiral stationary phases are based on low-molecular-weight selectors.

We focus our attention on novel **polymer** based stationary phase.

✓ Multiple dipole interaction ✓ Cooperative interaction ✓ High durability

2. Previous Work: Poly(butylene terephthalate) (PBT) Column Investigated under SFC Condition

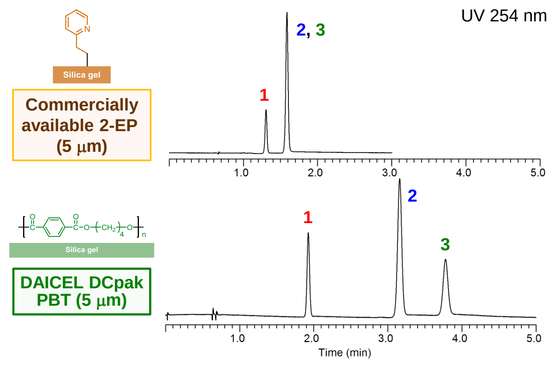


DAICEL DCpak PBT
(Particle size: 5 μm and 3 μm)

- ✓ **Unique planarity recognition of isomeric and closely similar samples.**
- ✓ **Orthogonal retention relationship of 2-ethylpyridine (2-EP) and this columns was attained.**
- ✓ **Column robustness was confirmed by using a variety of modifiers and by cycle durability testing.**

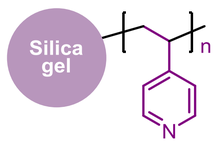
K. Nagai, T. Shibata, S. Shinkura, A. Ohnishi, *J. Chromatogr. A* **2018**, *1549*, 85–92.

Terphenyl isomers



Column: 4.6 x 150 mm, Eluent: **MeOH (3% isocratic)**, Flow: 3 ml/min, Temp.: 40 °C, BPR: 15.0 MPa

3. Poly(4-vinylpyridine) (P4VP) Column Investigated under SFC Condition

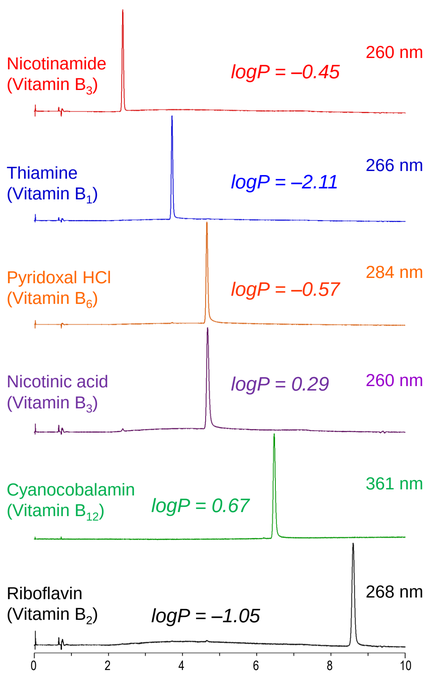


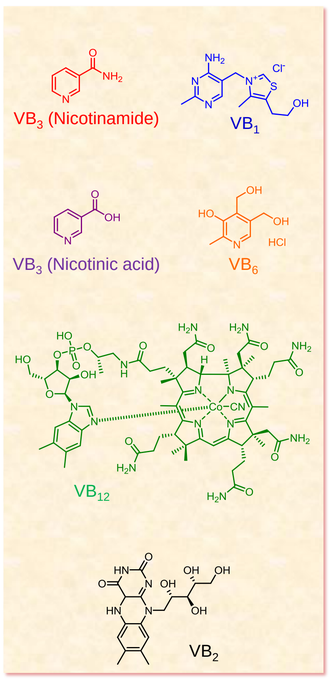
DAICEL DCpak P4VP
(Particle size: 5 μm and 3 μm)

K. Nagai, T. Shibata, S. Shinkura, A. Ohnishi, *submitted.*

✓ **Versatile Column**
P4VP column can be applied from non-polar to polar samples.

Water Soluble Vitamins

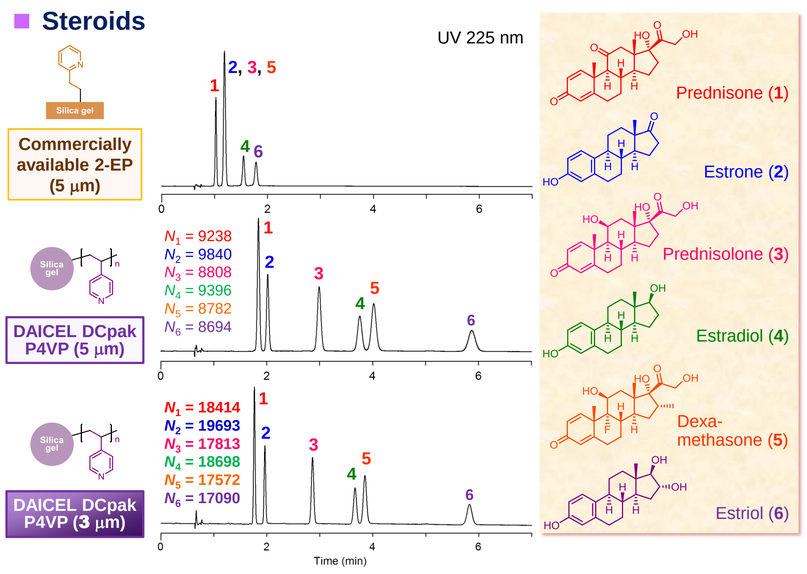




Column: 4.6 x 150 mm, 5 μm, Flow: 3 ml/min, Temp.: 40 °C, BPR: 15.0 MPa. Eluent: MeOH + **20 mM ammonium formate (10% to 60% gradient in 10 min)**.

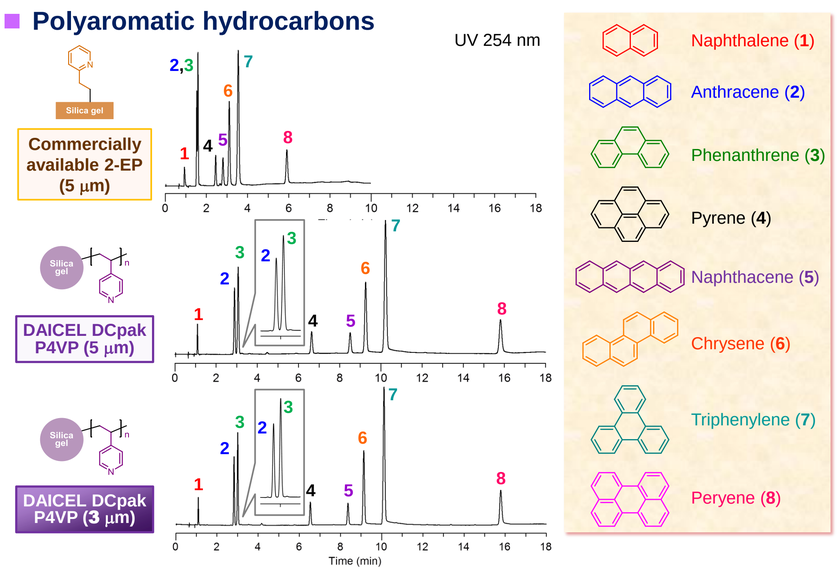
✓ **High Planarity Recognition**
P4VP Column shows excellent planarity recognition and molecular shape recognition.

Steroids



Column: 4.6 x 150 mm, Eluent: MeOH (30% isocratic), Flow: 3 ml/min, Temp.: 40 °C, BPR: 15.0 MPa

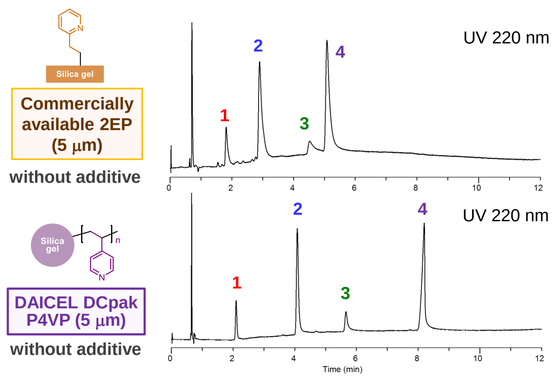
Polyaromatic hydrocarbons



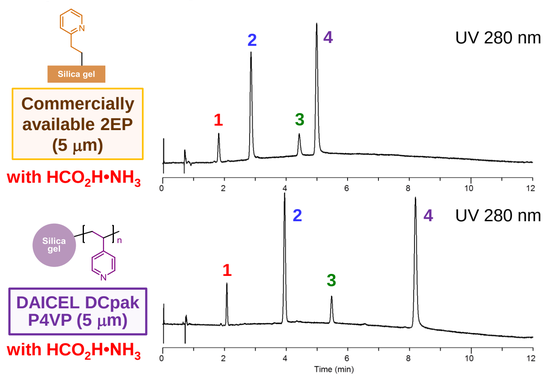
Column: 4.6 x 150 mm, Eluent: **MeOH gradient (3 to 38%)**, Flow: 3 ml/min, Temp.: 40 °C, BPR: 15.0 MPa

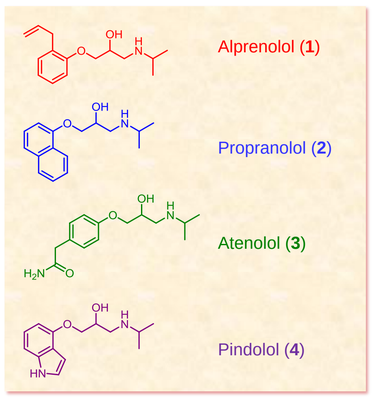
✓ **Good Peak Shape without Additive**
P4VP column can afford symmetrical peaks even in the absence of any additives for APIs.

β-Blockers (without additive)



β-Blockers (with 20 mM ammonium formate)





Column: 4.6 x 150 mm, 5 μm, Eluent: **MeOH + 20 or 0 mM ammonium formate gradient (10 to 35% in 10 min)**, Flow: 3 ml/min, Temp.: 40 °C, BPR: 15.0 MPa

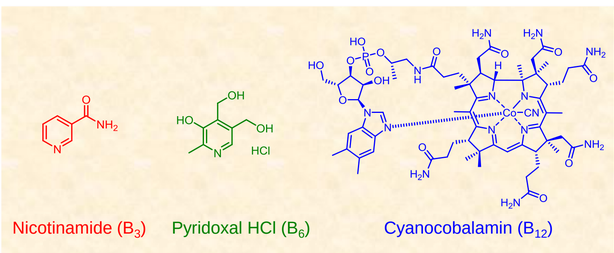
✓ **Durability Testing**
Column robustness of P4VP column was confirmed by cycle durability testing.

Experiment

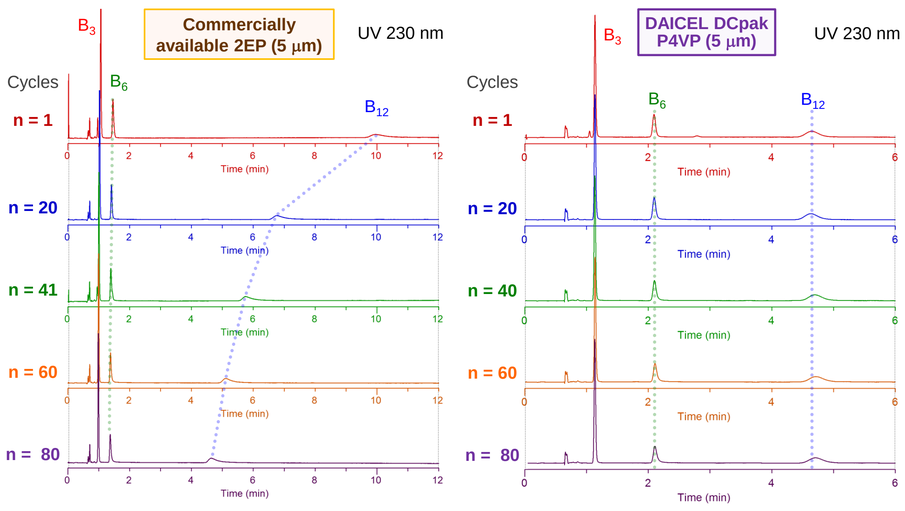
Flow rate: 3.0 ml/min, CO_2/MeOH = 75/25, 40 °C, BPR: 15 MPa, Time: 15 min.

Run over same analysis for 80 cycles.

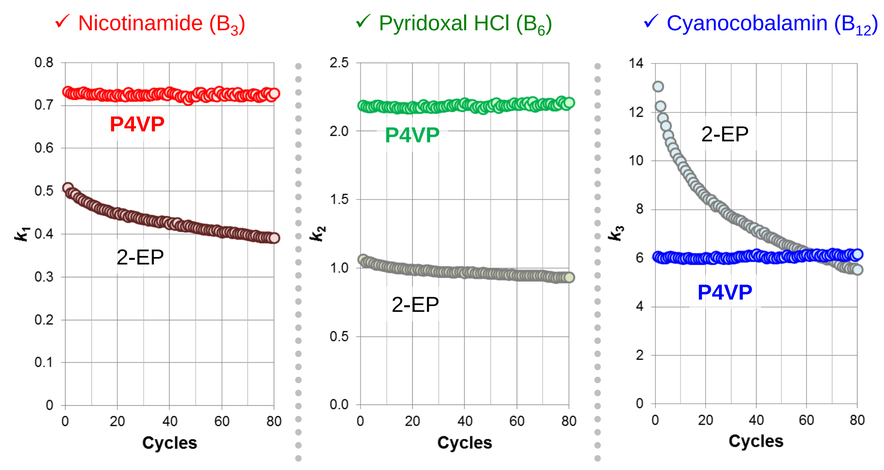
- We used newly purchased columns and CO_2 cylinders.



Cycle Dependent SFC Chromatogram



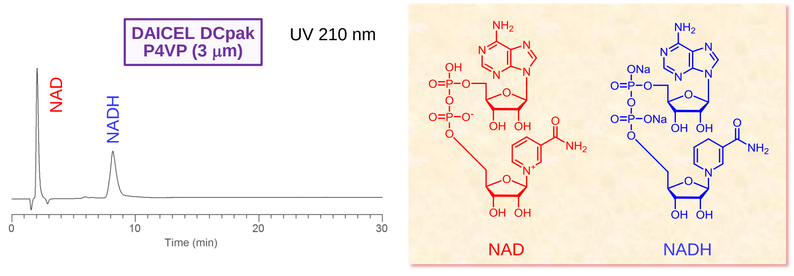
Cycle vs Retention Factor (*k*)



4. P4VP Column Investigated under HPLC Modes

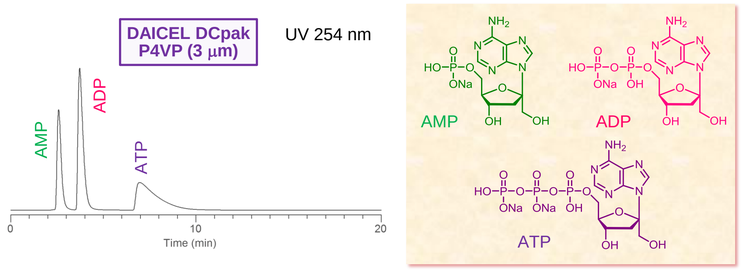
HPLC application dealing with hydrophilic samples was conducted.

NAD, NADH



Column: 2.1 x 150 mm, 3 μm, Flow: 0.3 ml/min, Temp.: 40 °C, Eluent: CH_3CN/ammonium acetate buffer (pH 4.8) = 40/60 (isocratic).

AMP, ADP, ATP



Column: 2.1 x 150 mm, 3 μm, Flow: 0.3 ml/min, Temp.: 40 °C, Eluent: CH_3CN/10 mM phosphate buffer (pH 7.0) = 10/90 (isocratic).

5. Conclusion

- ✓ **A novel P4VP based column was designed and its performance was evaluated under SFC conditions.**
- ✓ **This column showed unique molecular shape recognition for planar molecules.**
- ✓ **P4VPcolumn afforded symmetric peaks for APIs probably due to the effective shield of residual silanols by polymeric pyridine selector.**
- ✓ **Stability towards cycle durability was confirmed.**
- ✓ **Application in HPLC mode was attained.**

We would like to appreciate Mr. Jun Zhu and Ms Yanlin Wang in DAICEL Chiral Technologies (China) for their help in HPLC analysis.