

# Analysis of Hydrophilic Molecules in Biological Fluid with Novel Mixed-Mode Column

Ken Tseng, Scott Silver, and Yuichi Fusho, Shodex

In pharmaceutical development, it is important to analyze small molecules or their metabolites in biological fluids. For this purpose, the analytical methods such as sample pretreatment, 2D-LC and LC-MS have been developed. However there are still problems of resolution and protein adsorption. As a result, satisfying analytical results have not always been achieved.

To solve such problems, we have developed a unique polymer based HPLC column having mixed-mode of size-exclusion and reversed-phase, ODP2 HP series.

The ODP2 HP Series provides the following:

- Better retention of polar substances under reversed phase mode
- Higher theoretical plate number
- Increased recovery of protein
- Lower salt concentration requirement in mobile phase
- Improved stability in high alkaline solution

In this report, we introduce:

- The retention data of polar substances compared with conventional ODS columns
- The application of pharmaceutical molecule analysis in biological fluids

## Experimental Conditions

### 1) The retention of highly polar substances

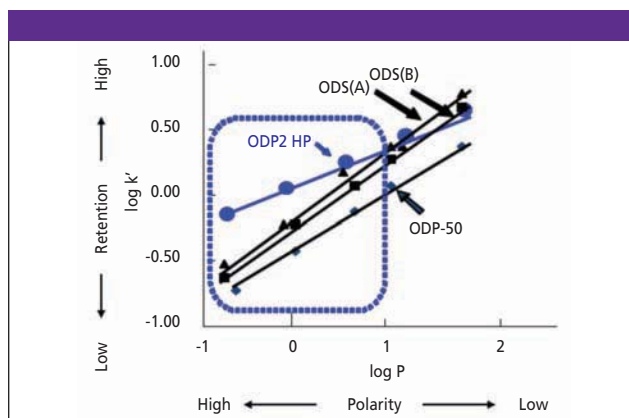
Shodex ODP2 HP-4D, Shodex Asahipak ODP-50 4D, ODS columns made by company A and B were used. The size of each column was 150 mm × 4.6 mm. The eluent was run with 75% water and 25% acetonitrile, 1.0 mL/min, at 40° C. The relationship between the hydrophobic parameter (log P) and retention performance (log k') was obtained through using uracil, theobromine, caffeine, pyridine and 2-methyl pyridine.

### 2) The analysis of pharmaceutical molecules in biological fluid

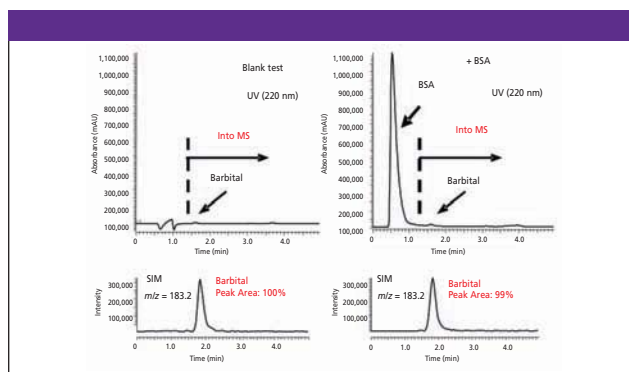
Shodex ODP2 HP-2B (50 mm × 2.0 mm) was used. The sample solution contained 7.0 mg/L of Bovine Serum Albumin (BSA) and 500 µg/L of barbital (or phenobarbital, hexobarbital). The Eluent was 10mM CH<sub>3</sub>COONH<sub>4</sub> aq./CH<sub>3</sub>CN = 70/30, flow rate = 0.2mL/min, at 30° C. The fraction of small molecules was then introduced into ESI-MS by column switching.

**Table I: Specification of ODP2 HP series columns. They are packed with macroporus poly(hydroxymethacrylate), and it is operational between pH 3 to 12.**

Product	Theoretical plate (per column)	Particle size (µm)	ID x L (mm)
ODP2 HP-4B	≥ 3,000	5	4.6 x 50
ODP2 HP-4D	≥ 10,000	5	4.6 x 150
ODP2 HP-4E	≥ 17,000	5	4.6 x 250
ODP2 HPG-4A	guard column	5	4.6 x 10
ODP2 HP-2B	≥ 2,500	5	2.0 x 50
ODP2 HP-2D	≥ 7,000	5	2.0 x 150
ODP2 HPG-2A	guard column	5	2.0 x 10



**Figure 1:** ODP2 HP shows stronger retention of highly polar substances compared with other conventional ODS columns and ODP-50. It is packed with macroporus poly(hydroxymethacrylate), and it is operational between pH 3 to 12. It can separate both polar and non-polar small molecules.



**Figure 2:** Barbital (target molecule) was separated from BSA cleanly and was detected by LCMS without showing ion suppression.

## Results and Conclusions

- 1) We have developed a unique polymer based HPLC column with mixed-mode of size-exclusion and reversed-phase, ODP2 HP series (Table I)
- 2) ODP2 HP can retain highly polar substances compared with conventional ODS columns
- 3) In the direct LC-MS analysis of small molecules in biological fluid, ODP2 HP columns provide successful separation of polar molecules from protein, and avoided ion suppression to produce good sensitivity in the ESI-negative mode.

MD Scientific is authorised distributor in Denmark for Shodex  
www.md-scientific.dk - 7027 8565 - info@md-scientific.dk