Shodex™

HPLC Columns

Shodex™ ODP2 HP series columns

Better retention of highly polar substances

Technical notebook
No. 6

SHOWA DENKO EUROPE

Contents

1. Introduction

The Shodex ODP2 HP series offers polymer–based columns for reversed phase chromatography. The efficiency of ODP2 HP columns is improved over most resin-based columns and is comparable with that of silica-based ODS columns.

ODP2 HP has a better retention of highly polar substances compared to most general purpose ODS columns. ODS columns are susceptible to protein adsorption, resulting in degradation of the column. ODP2 HP is designed to exclude protein and thus, ODP2 HP can be used for analysis of drugs in biological samples containing protein without rapid column deterioration. As ODP2 HP can be used with low salt concentration without loss of peak shapes, it is excellent for LC/MS analysis.

1-1. Specifications

Table 1. Specification of ODP2 HP Series Columns

Product Code	Product Name	TPN (per column)	Particle Size (μm)	ID x Length (mm)
F7622001	ODP2 HP-4B	≥ 3,500	5	4.6 x 50
F7622002	ODP2 HP-4D	≥ 13,000	5	4.6 x 150
F7622003	ODP2 HP-4E	≥ 17,000	5	4.6 x 250
F6714010	ODP2 HPG-4A	Guard Column	5	4.6 x 10
F7622004	ODP2 HP-2B	≥ 3,000	5	2.0 x 50
F7622005	ODP2 HP-2D	≥ 7,000	5	2.0 x 150
F6714011	ODP2 HPG-2A	Guard Column	5	2.0 x 10

For all Columns

Packing Material : Macroporous Poly(hydroxymethacrylate) Particles
Housings : 316 Stainless Steel
In-column Solvent (Initial) : Water/Acetonitrile = 55/45
pH Range : 3~12
Temperature : 20~60℃
Eluent Compatibility : Please refer to section 1-2.

1-2. Eluent Compatibility of ODP2 HP Series

ODP2 HP may be used with water, acid, base and aqueous salt solutions including most popular buffers, acetonitrile, methanol and mixtures of these components.

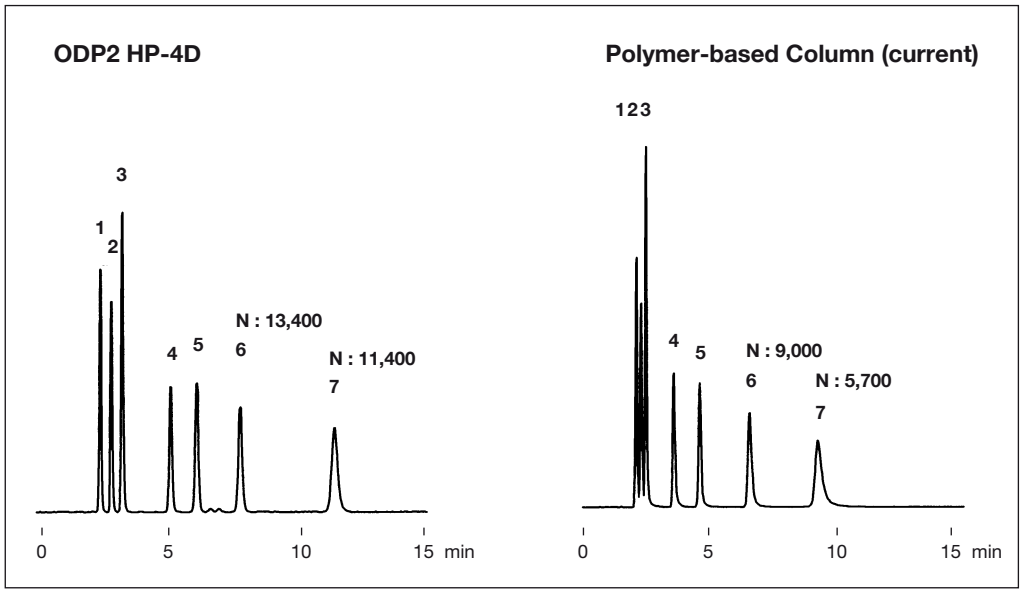
<Representative Acids> Phosphoric Acid, Formic Acid, Acetic Acid, and Trifluoroacetic Acid
<Representative Bases> Ammonia
<Representative Buffers> Phosphate Buffer, Formate Buffer, Acetate Buffer, and Carbonate Buffer
<Polar Organic Solutions> Methanol, Acetonitrile

(Precautions)
1) Eluent should be in the pH range of 3~12.
2) The total concentration of acid, base, and salt should be 100mM or less. Generally, a range of 1~50mM is recommended.
3) When adding acetonitrile or methanol to the aqueous salt solution, confirm there is no salt precipitation before use.
4) Nonpolar organic substances such as hexane or toluene cannot be used.

2. Advantages of ODP2 HP

2-1. High Efficiency

Chromatograms of ODP2 HP-4D and ODP-50 are shown in Fig. 2-1. ODP-50 4D is a popular polymer-based column for reversed phase separation. The theoretical plate number (TPN) of ODP2 HP-4D, measured with naphthalene, is nearly double that of the current column, ODP-50 4D.



Sample: 5μL

1. Uracil	30mg/L
2. Theobromine	75mg/L
3. Caffeine	130mg/L
4. Phenol	300mg/L
5. Methyl Benzoate	350mg/L
6. Toluene	1000mg/L
7. Naphthalene	150mg/L

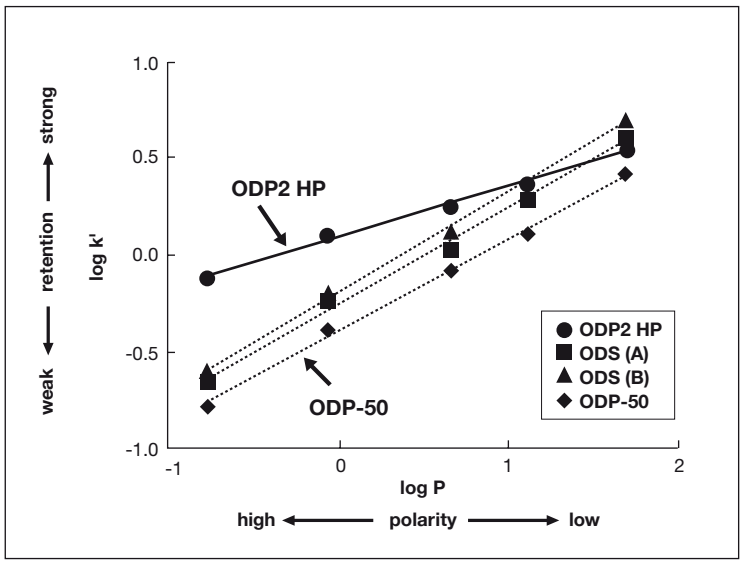
Fig. 2-1 Comparison of ODP2 HP and Current Column

Column : Shodex ODP2 HP-4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN=55/45
Flow Rate : 0.6mL/min
Detector : UV (254nm)
Column Temp. : 40℃

Column : Shodex Asahipak ODP-50 4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN=35/65
Flow Rate : 0.6mL/min
Detector : UV (254nm)
Column Temp. : 40℃

2-2. Retention of Highly Polar Compounds

Figure 2-2 shows the relation between the hydrophobic parameter (log P) and the retention performance (log k'). ODP2 HP showed stronger retention of highly polar substances compared to other ODS columns and ODP-50.



* The smaller the value of log P, the higher the polarity; the higher the value of log k', the higher the retention performance.

Column : Shodex ODP2 HP-4D, Shodex Asahipak ODP-50 4D ODS(A), ODS(B) (4.6mmID x 150mm each)
Eluent : H_2O/CH_3CN=75/25
Flow Rate : 1.0mL/min
Column Temp. : 40℃

Fig. 2-2 Relation between Hydrophobic Parameter and Retention

2-3. Simple Analysis of Basic Substances

Reversed phase chromatography is generally performed under conditions either with suppressing dissociation of the sample or with an ion-pair reagent, of opposite charge to the sample, added to the mobile phase. This allows separation based on hydrophobicity. However, analysis using ion pair reagents is rather complex due to the apparent involvement of two separation modes as shown below. In addition, the column once used with the ion pair reagent is not generally reusable for different analyses as the reagent is adsorbed to the column.

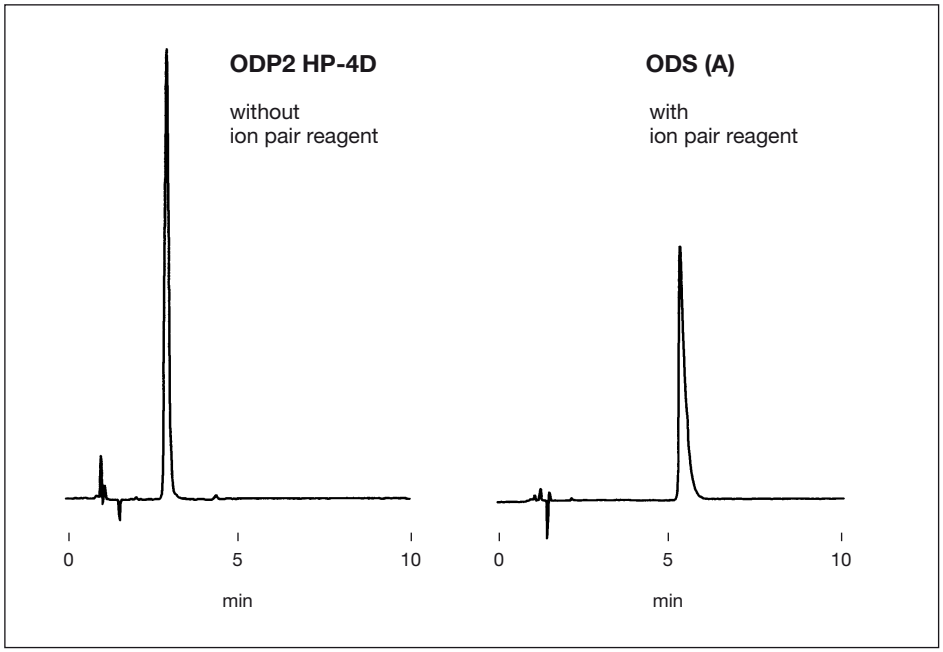
(1) Ionic sample and an ion pair reagent form ion pairs, which enhances hydrophobicity of the sample, and thus retention. Analysis of basic ionic substances can be described in the following:

$R\text{-}NH_3^{\oplus}$ + ion pair reagent$^{\ominus}$ $\Leftrightarrow$ [$R\text{-}NH_3^{\oplus\ominus}$ ion pair reagent]
basic substances ion pair

(2) Hydrophobic segment of an ion pair reagent is adsorbed to the reversed phase column, which will then act as an ion exchange column.

In the case of reversed phase analysis of basic compounds, such as short amines, analysis under alkaline conditions, which prevent basic substances from dissociating, is appropriate. However, because silica-based columns usually exhibit very short lifetimes in alkaline conditions, ion pair reagents are used for the analysis of basic compounds. On the contrary, ODP2 HP, a polymer-based reversed phase column, is superior in alkali durability*. In other words, it is possible to operate in alkaline eluent and simplify the reversed phase separation of basic compounds by using ODP2 HP. Eliminating a need for ion-pair reagents enhances both the separation process and the detection process for basic compounds. Figure 2-3 shows examples of basic drug analysis using the ODP2 HP and ODS column, ODS (A)**.

* Please refer to section 2-7 concerning alkali durability of ODP2 HP columns.
** ODS(A) has better end-capping of residual silanol groups than ODS(B).



Sample: 5μL
Atropine(pKa 9.9) 300mg/L

Fig. 2-3 Analysis of Basic Substances by ODP2 HP and ODS(A)

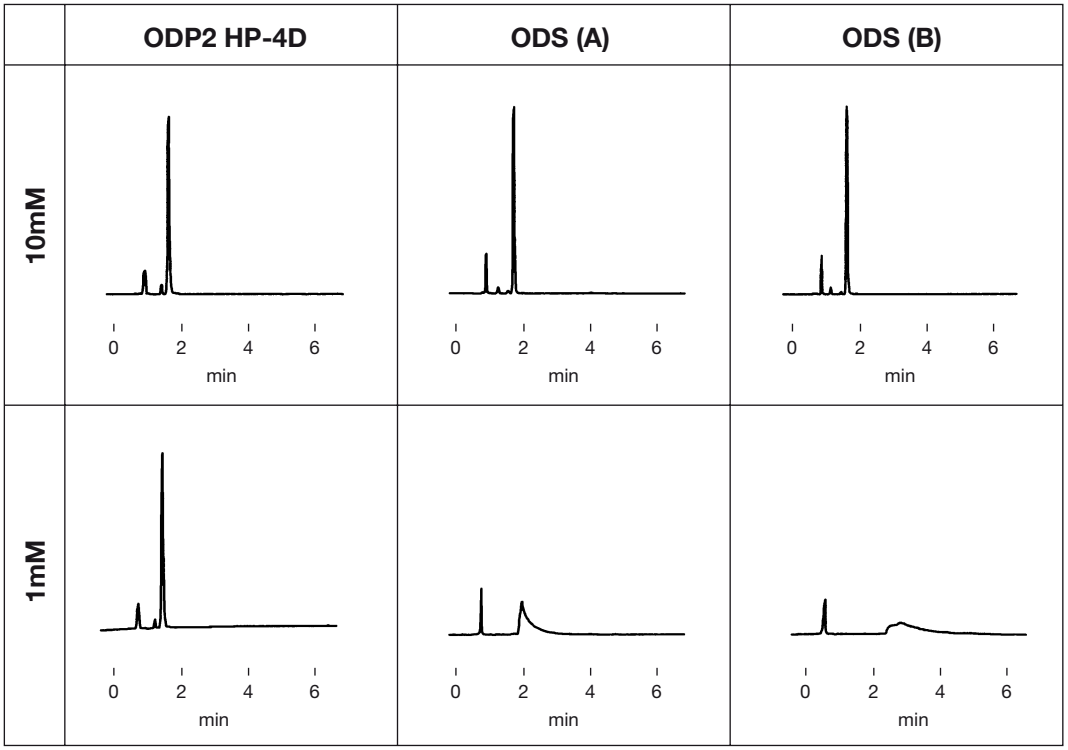
Column	: Shodex ODP2 HP-4D (4.6mmID x 150mm)	Column	: ODS(A) (4.6mmID x 150mm)
Eluent	: 10mM Sodium Phosphate Buffer (pH11) /CH_3CN=55/45	Eluent	: 0.1% 1-Pentanesulfonic Acid Sodium Salt /CH_3CN=55/45
Flow Rate	: 1.0mL/min	Flow Rate	: 1.0mL/min
Detector	: UV (220nm)	Detector	: UV (220nm)
Column Temp.	: 40˚C	Column Temp.	: 40˚C

2-4. Ability for Use with Low Salt Concentration

The relationship between ammonium acetate concentration and separation performance was compared between ODP2 HP-4D and two ODS columns as shown in figure 2-4. When a 10mM ammonium acetate buffer was used as an eluent, each column showed a good chromatogram. When the ammonium acetate concentration was lowered to 1mM, both ODS columns showed tailing peaks caused by interaction between scopolamine and the residual silanol groups in the column. ODS(A)** which has better end-capping of residual silanol groups than ODS(B), still shows the influence of some residual silanol groups.

On the other hand, polymer based ODP2 HP-4D showed no non-specific adsorption of scopolamine to the media even when the ammonium acetate concentration was lowered. Scopolamine elutes with a sharp peak.

As elution time and peak shapes are not affected even if the salt concentration in the eluent is lowered, ODP2 HP columns are suitable for ESI methods (LC/MS) where salt concentration in the eluent affects ion suppression of the sample.



Sample: 5μL
Scopolamine (pKa7.6)
300mg/L

Fig. 2-4 Relation between Ammonium Acetate Concentration and Separation Performance

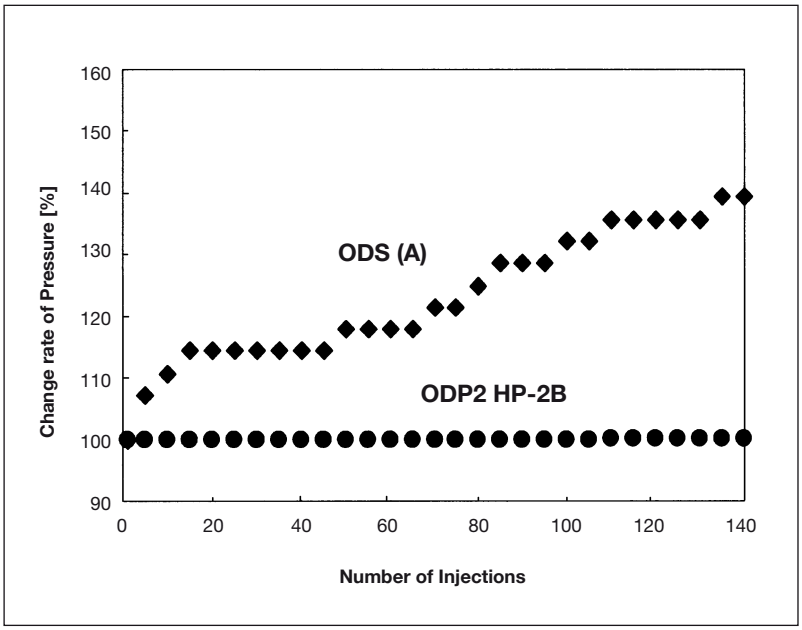
Column : Shodex ODP2 HP-4D, ODS(A), ODS(B) (4.6mmID x 150mm each)
Eluent : CH_3COONH_4 Buffer (pH7.0)/CH_3CN=35/65
Flow Rate : 1.0mL/min
Detector : UV (220nm)
Column Temp. : 40˚C

** ODS(A) has better end-capping of residual silanol groups than ODS(B).

2-5. Capability for Protein Elimination

Generally, protein is adsorbed to ODS columns when injected, and is a cause of column degradation. ODP2 HP media has high polarity and small pores, which prevent protein adsorption. Protein is almost completely excluded from the column and not adsorbed to the column.

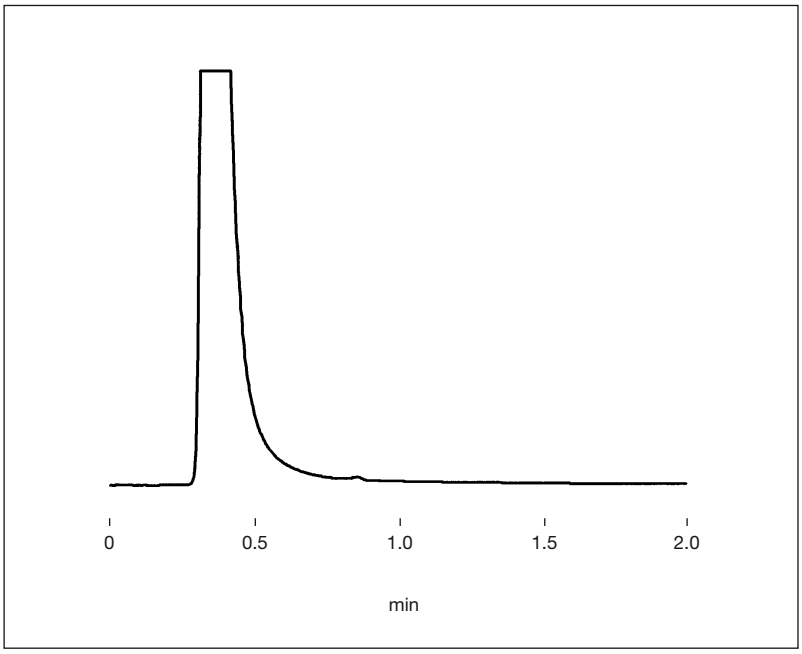
The relationship between the number of BSA injections and pressure change rate is shown in figure 2-5. The chromatogram of bovine serum albumin (BSA) is shown in figure 2-6. ODS (A) showed a drastic pressure increase with repeated injections of BSA, because BSA was adsorbed to the media in the column. However, ODP2 HP-2B showed stable pressure even after the 140th injection of BSA as BSA was eluted early as shown in



Sample: 5μL
BSA 7.0mg/mL

Column : Shodex ODP2 HP-2B, ODS(A) (2.0mmID x 50mm each)
Eluent : 1mM CH_3COONH_4 aq. /CH_3CN=90/10
Flow Rate : 0.2mL/min
Detector : UV (220nm)
Column Temp. : 30℃

Fig. 2-5 Relation between Number of BSA Injections and Pressure Rate Change



Sample: 5μL
BSA 7.0mg/mL

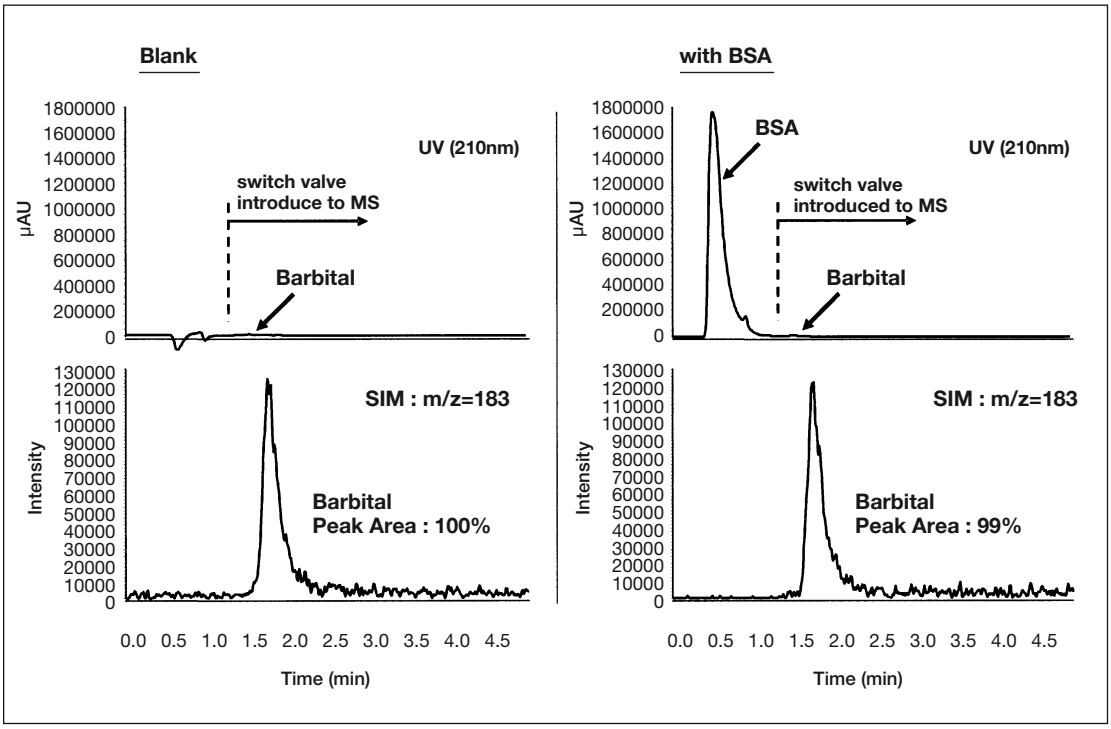
Column : Shodex ODP2 HP-2B (2.0mmID x 50mm)
Eluent : 1mM CH_3COONH_4 aq. /CH_3CN=90/10
Flow Rate : 0.2mL/min
Detector : UV (220nm)
Column Temp. : 30℃

Fig. 2-6 Chromatogram of BSA

2-6. Application for the Analysis of Drugs in Biological Fluid

LC/MS is effective for the high sensitivity analysis of drugs; however, when protein is present and enters the MS (mass detector), it contaminates the MS or suppresses ionization of the sample. Often pretreatment does not remove protein thoroughly. Drugs in biological fluid are hard to analyze because protein co-elutes with the component of interest. The target drug receives ion suppression from the protein and appears as a small peak.

As discussed in chapter 2.5 “Capability for Protein Elimination”, ODP2 HP can separate the target from protein by eluting protein early and cleanly. The result of barbital (drug) analysis with BSA using LC/MS is shown in figure 2-7. Barbital was introduced into the MS by a switching valve after BSA (protein) was eluted, and barbital was detected without any influence of ion suppression.



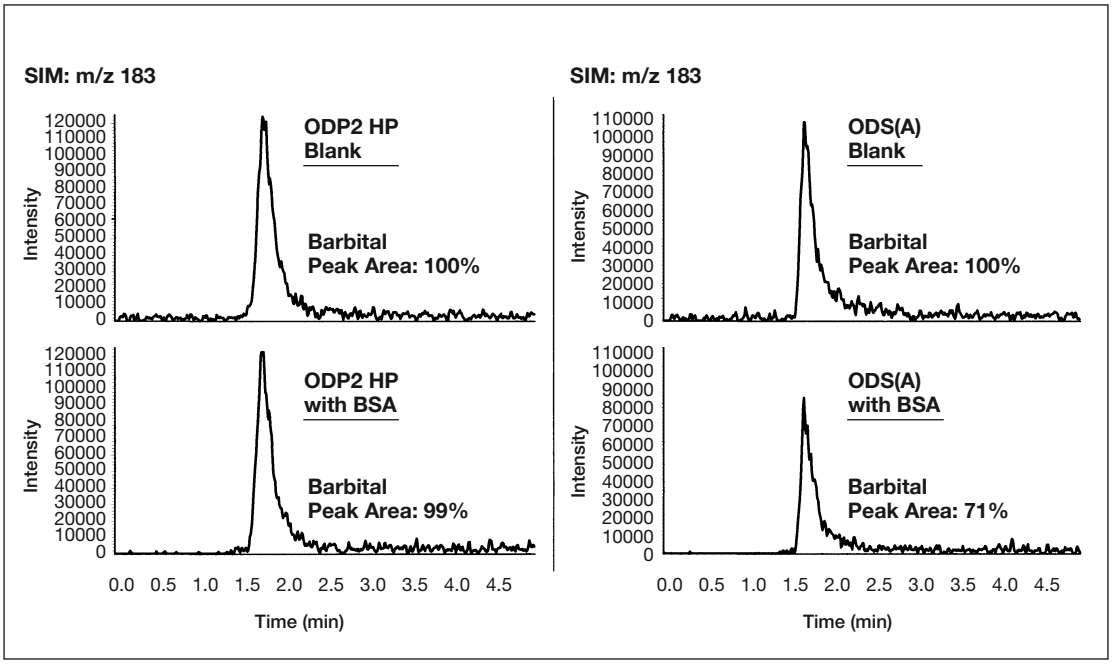
Sample: 5μL
Barbital 500μg/L
BSA 7.0mg/mL

Fig. 2-7 Analysis of Barbital in BSA (LC/MS)

Column : Shodex ODP2 HP-2B (2.0mmID x 50mm)
Eluent : 10mM CH_3COONH_4 aq./CH_3CN=70/30
Flow Rate : 0.2mL/min
Detector : UV (210nm), ESI-MS (SIM Negative)
Column Temp. : 30℃

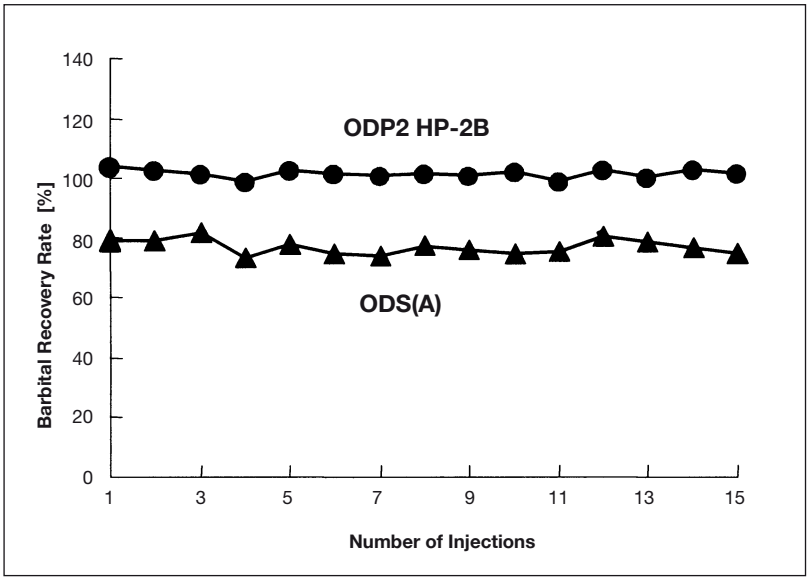
Four LC/MS chromatograms of barbital analysis are shown in figure 2-8 along with barbital recovery rates in figure 2-9. These compare ODP2 HP-2B and a well end-capped ODS column, ODS (A)**, for the detection of barbital without BSA and with BSA. ODP2 HP-2B showed good separation of protein and drug, with little ion suppression effect and high barbital recovery rate even after repeated injections. On the other hand, ODS (A) with BSA showed smaller barbital peak and lower recovery rate compared with ODS (A) without BSA due to ion suppression caused by protein.
As you can see, ODP2 HP is well suited for LC/MS analysis of drugs in biological fluid.

** ODS(A) has better end-capping of residual silanol groups than ODS(B).



Sample: 5μL
Barbital 500μg/L
BSA 7.0mg/mL

Fig. 2-8 Recovery Rate of Barbital in BSA (LC/MS)



Column : Shodex ODP2 HP-2B, ODS(A)
(2.0mmID x 50mm each)
Eluent : 10mM CH_3COONH_4 aq.
/CH_3CN=70/30
Flow Rate : 0.2mL/min
Detector : ESI-MS (TIC)
Column Temp. : 30℃

Fig. 2-9 Recovery Rate of Barbital in BSA with Additional Injections

2-7. Alkali Durability

Generally, silica-based ODS columns degrade rapidly when an alkali eluent is used, because the silica gel substrate is dissolved under alkaline conditions. The chromatograms before and after use of alkali eluent were compared for ODP2 HP-4D and ODS(A), as shown in figure 2-10. Also, the relative theoretical plate number (TPN) for pyridine and flow duration is shown in figure 2-11. TPN before flowing alkali eluent is set to 100% in this test. For ODS (A) retention times of each sample decreased rapidly, reducing the theoretical plate number after 24 hours of flowing alkali eluent, thus showing degradation of the column. On the other hand, the retention times of each sample and TPN were virtually unchanged even after 500 hours of flowing alkali eluent. This shows the superiority of ODP2 HP in alkali durability.

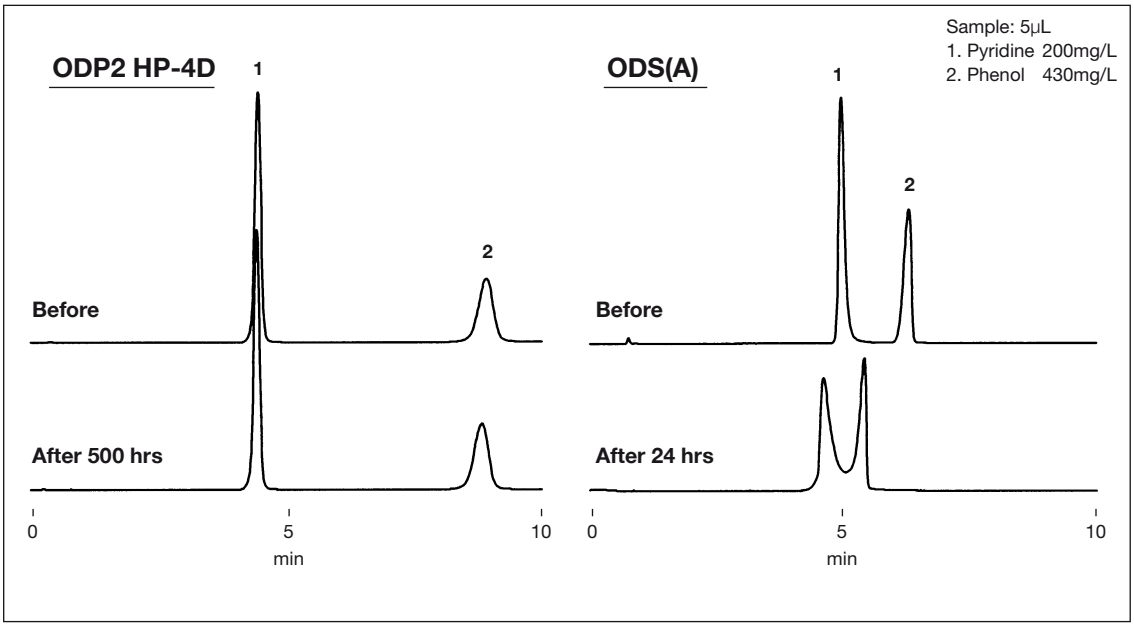
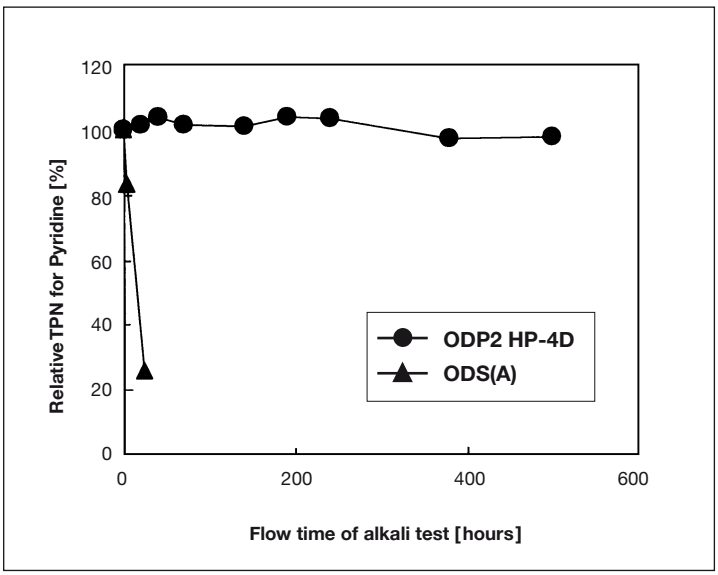


Fig. 2-10 Comparison of Chromatograms before and after Alkali Test



Test Condition of Alkali Durability

Column	: Shodex ODP2 HP-4D, ODS(A) (4.6mmID x 150mm each)
Eluent	: 10mM Phosphate Buffer(pH12) /CH_3CN=45/55
Flow Rate	: 0.6mL/min
Column Temp.	: 30℃

Analysis Condition

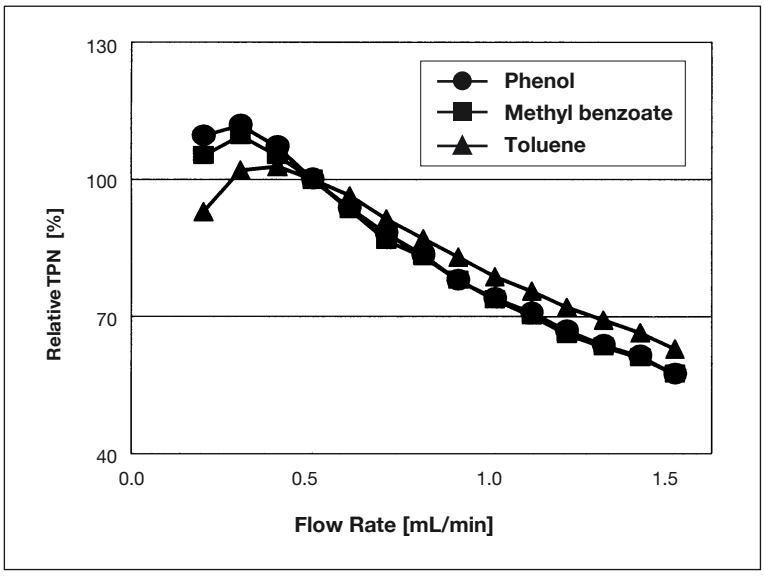
Column	: Shodex ODP2 HP-4D, ODS(A) (4.6mmID x 150mm each)
Eluent	: H_2O/CH_3OH=70/30
Flow Rate	: 1.0mL/min
Detector	: UV (254nm)
Column Temp.	: 40℃

Fie. 2-11 Relation between Flow Time of Alkali Test and Relative TPN

3. Performance of ODP2 HP Series

3-1. Influence of Flow Rate

The relation between the theoretical plate number (TPN) and flow rate for ODP2 HP is shown in figure 3-1, and that of retention time and flow rate is shown in figure 3-2. The data refers to ODP2 HP-4D column (4.6mm ID x 150mm length). Each sample showed the highest theoretical plate number (TPN) at the flow rate of 0.4mL/min, while efficiency decreased at 0.3mL/min and below due to sample diffusion. Also, each sample showed extremely long retention times at 0.5mL/min and below. Therefore a flow rate of 0.5 - 1.0mL/min is recommended for general analysis using ODP2 HP-4D. Flow rates of 0.1 - 0.2mL/min are recommended for ODP2 HP-2D (2mm ID x 150mm length),



Sample: 5μL
1. Phenol 300mg/L
2. Methyl benzoate 350mg/L
3. Toluene 1000mg/L

Fig. 3-1 Relation between Efficiency (TPN) and Flow Rate

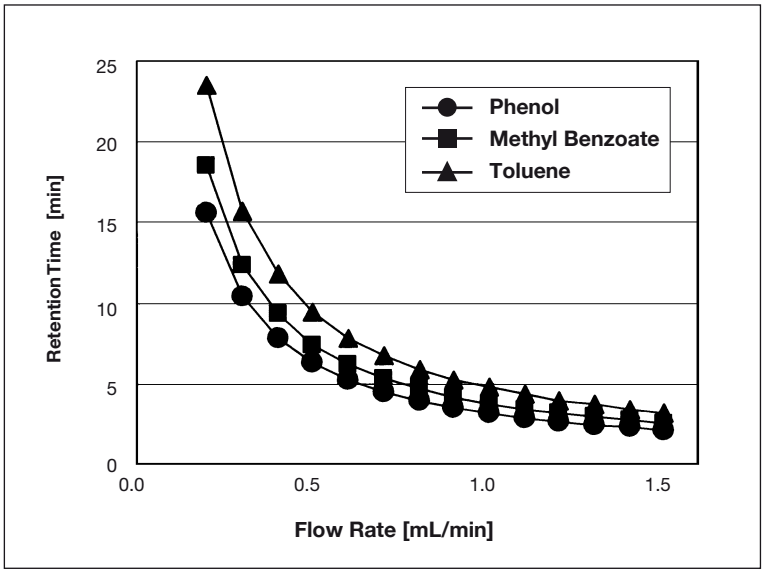
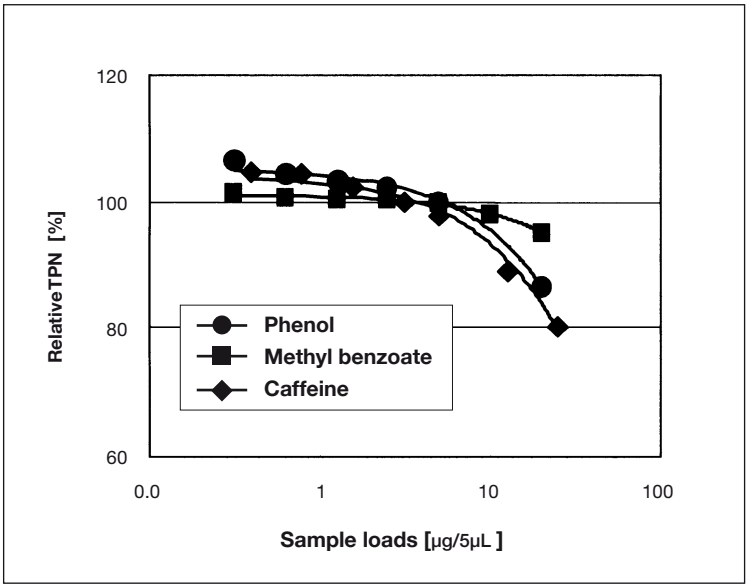


Fig. 3-2 Relation between Retention Time and Flow Rate

Column : Shodex ODP2 HP-4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN=55/45
Detector : UV (254nm)
Column Temp. : 40℃

3-2. Influence of Sample Loads

The relation between sample loads and theoretical plate number (TPN) for ODP2 HP-4D (4.6mm ID x 150mm length) is shown in figure 3-3. Sample loads of 10μg or less are recommended with ODP2 HP-4D for best column performance and sample loads of 2 μg or below are recommended for ODP2 HP-2D (2mm ID x 150mm length).



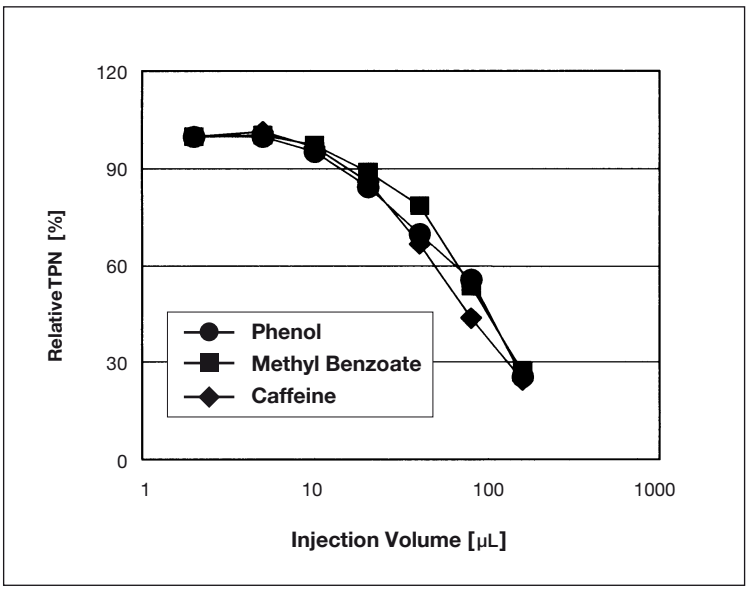
Sample : 5μL
1. Phenol
2. Methyl benzoate
3. Caffeine

Column : Shodex ODP2 HP-4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN=55/45
Flow rate : 1.0mL/min
Detector : UV (254nm) Phenol, Methyl benzoate
UV (300nm) Caffeine
Column temp. : 40˚C

Fig. 3-3 Relation between sample loads and TPN

3-3. Influence of Sample Injection Volume

The relation between sample injection volume and theoretical plate number for ODP2 HP-4D (4.6mm ID x 150mm length) is shown in figure 3-4. Sample injection volume of 40μL and below is recommended for ODP2 HP-4D to achieve maximum efficiency. A sample injection volume of 8μL and below is recommended for ODP2 HP-2D (2mm ID x 150mm length).



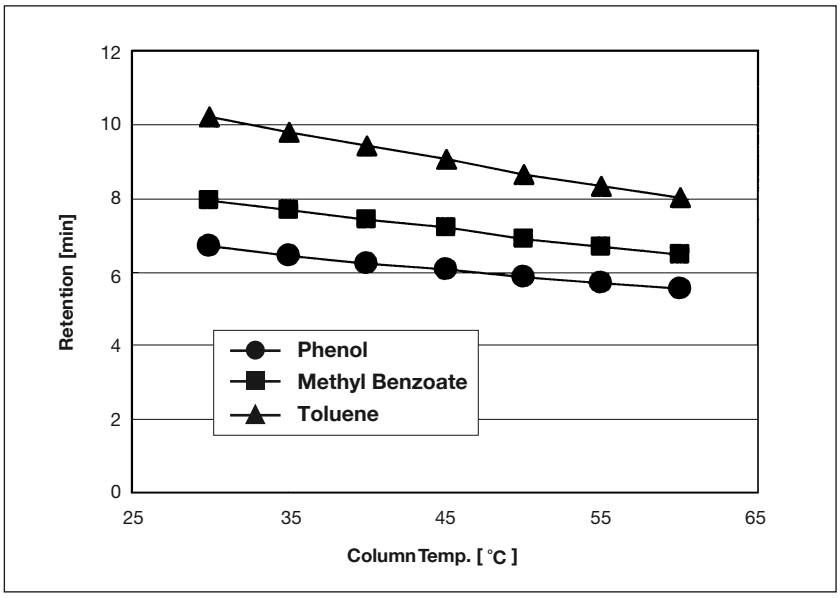
Sample: 0.6μg each
1. Phenol
2. Methyl Benzoate
3. Caffeine

Column : Shodex ODP2 HP-4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN=55/45
Flow Rate : 1.0mL/min
Detector : UV (254nm) Phenol, Methyl Benzoate
UV (300nm) Caffeine
Column Temp. : 40˚C

Fig. 3-4 Relation between Sample Injection Volume and TPN

3-4. Influence of Temperature

The relation between column temperature and retention time is shown in figure 3-5, and the relation between column temperature and theoretical plate number (TPN) is shown in figure 3-6. As retention time and TPN vary with changes in column temperature, the use of a column oven for temperature control is recommended.



Sample: 5μL
1. Phenol 300mg/L
2. Methyl Benzoate 350mg/L
3. Toluene 1000mg/L

Fig. 3-5 Relation between Column Temperature and Retention Time

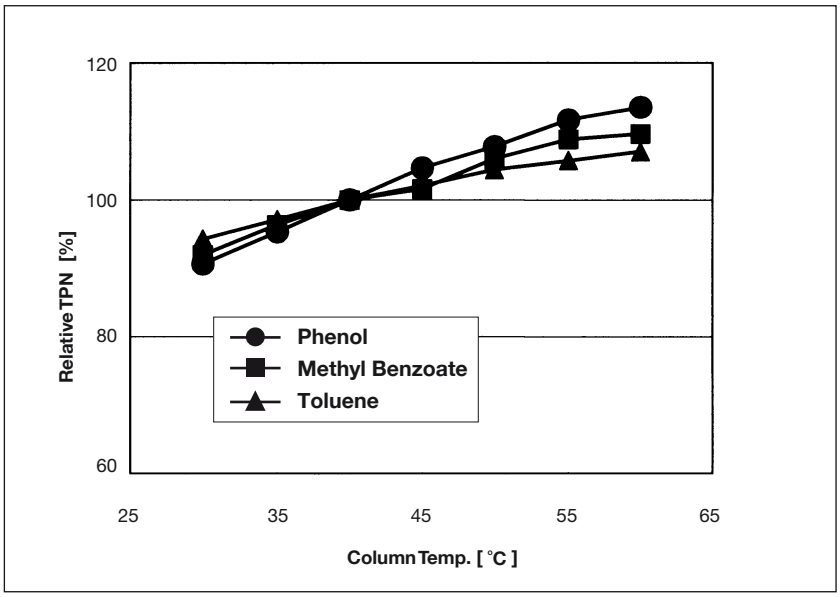
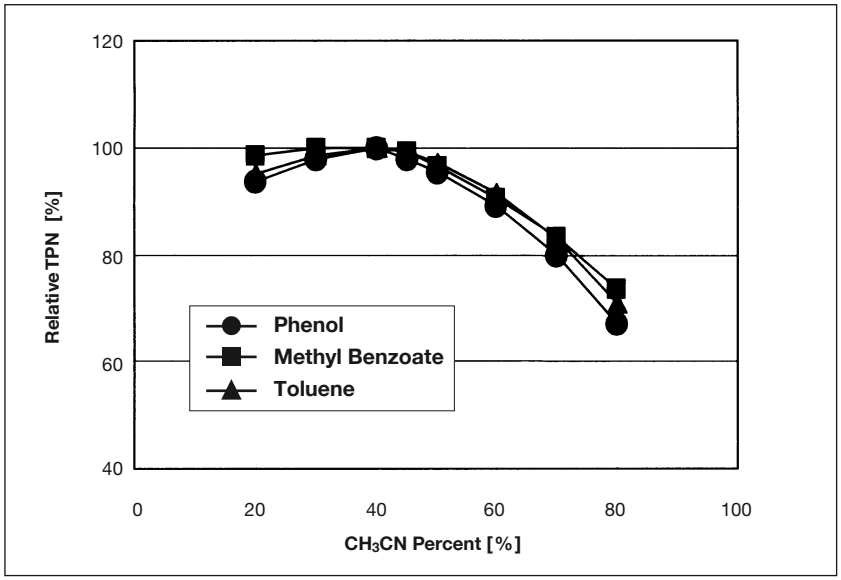


Fig. 3-6 Relation between Column Temperature and TPN

Column : Shodex ODP2 HP-4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN=55/45
Flow Rate : 0.5mL/min
Detector : UV (254nm)

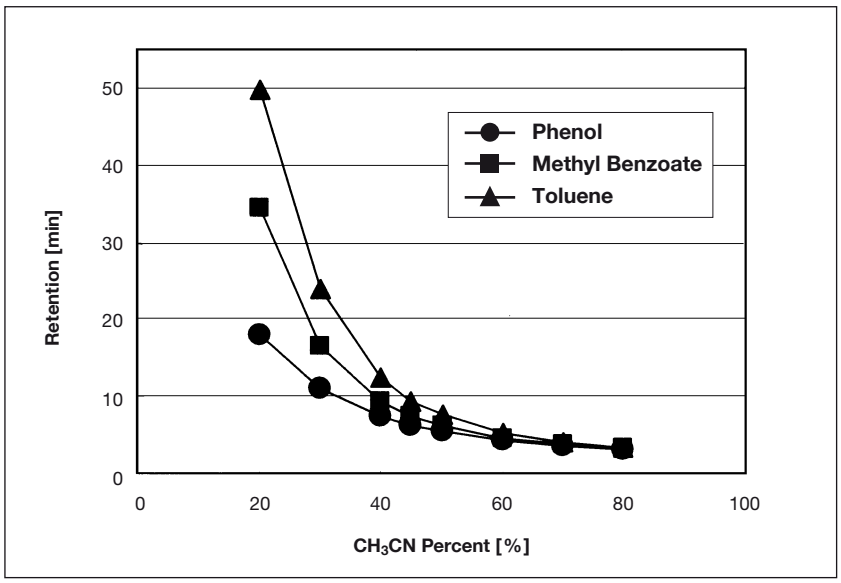
3-5. Influence of Organic Solvent in Eluent

The relation between the concentration of the organic solvent (acetonitrile) in the eluent and the theoretical plate number is shown in figure 3-7. For each sample the highest efficiency occurs when the content of organic solvent is 40 to 45%. The relation between the organic solvent (acetonitrile) in the eluent and the retention time of each sample is shown in figure 3-8. The retention time of each sample rapidly increased when the content of acetonitrile was 40% or below. A mobile phase of 45% acetonitrile is recommended as a starting point for general analyses.



Sample: 5μL
1. Phenol 300mg/L
2. Methyl Benzoate 350mg/L
3. Toluene 1000mg/L

Fig. 3-7 Relation between Concentration of Acetonitrile and TPN



Column : Shodex ODP2 HP-4D (4.6mmID x 150mm)
Eluent : H_2O/CH_3CN
Flow Rate : 0.5mL/min
Detector : UV (254nm)
Column Temp. : 40˚C

Fig 3-8 Relation between Concentration of Acetonitrile and Retention Time

4. Conclusions

The columns of the new ODP2 HP series demonstrate significant advantages for the analysis of high polarity substances. This notebook demonstrates the performance of the ODP2 HP series. Application data using ODP2 HP series will be introduced in another technical notebook.