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Application News

No. L549

High Performance Liquid Chromatography

Analysis of Cresol Positional Isomers by Using Shim-pack™ GIST Phenyl Column

Reversed phase chromatography (RPC), which is widely used in high performance liquid chromatography, is applicable to diverse compounds. The packing materials for columns used in RPC are substances that bond chemically with alkyl groups and other functional groups as a simple substance of silica gel. The octa decyl group (C18) and the octyl group (C8) may be mentioned as representative examples. The ODS (Octa Decyl Silyl) column is frequently used as the first choice when studying separation conditions, but separation of isomers is inadequate in some cases. Phenyl columns show different separation behavior from ODS columns. Due to the phenyl skeleton, in addition to the hydrophobic interaction, the π-π interaction also contributes to separation in the phenyl column, resulting in higher selectivity for aromatic compounds.

This article introduces an example of analysis of positional isomers of cresol by using a phenyl column that displays different separation characteristics from ODS columns among RPC columns.

A. Morita

Structures of Cresol Positional Isomers

Fig. 1 shows the structural formulas of cresol. Three types of positional isomers, ortho (*o*-), meta (*m*-), and para (*p*-), exist in cresol.

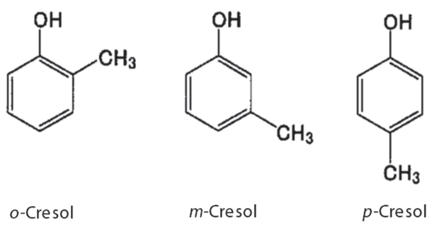


Fig. 1 Positional Isomers of Cresol

Features of Shim-pack Phenyl Column

Fig. 3 shows the lineup of the Shim-pack GIST Series and the features of the columns. The silica gel carrier of the Shim-pack GIST Series is high purity, completely porous spherical silica. Increased inertness not only improves analytical precision (peak shape), but also increases column durability. The Shim-pack GIST Phenyl column displays a π-π interaction owing to the phenyl skeleton. Because the phenyl group is bonded directly to the silica gel, the column has the characteristic that a larger difference in the electronic state of aromatic compounds can be recognized in comparison with general phenyl columns (alkyl phenyl group bonded columns).

Comparison of Separation by ODS Column and Phenyl Column

Fig. 2 shows the results of an analysis of standard solutions of *o*-, *m*-, and *p*-cresol (20 mg/L each) with a Shim-pack GIST Phenyl column and a Shim-pack GIST C18 column. Table 1 shows the analytical conditions. The linear velocity were matched and the organic solvent ratio was adjusted so that the retention time was approximately the same. The ODS column could not separate the *o*- and *m*-cresol. On the other hand, in the phenyl column, not only the hydrophobic interaction, but also the π-π interaction contribute to separation. As a result, selectivity for aromatic compounds is enhanced, and satisfactory separation could be achieved.

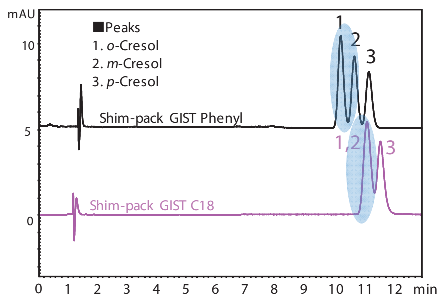


Fig. 2 Comparison of Chromatograms of Cresol Positional Isomers by Using Shim-pack GIST Phenyl (Top) and Shim-pack GIST C18 (Bottom)

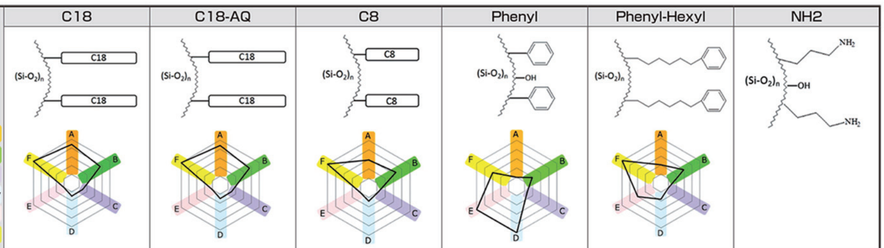
	C18	C18-AQ	C8	Phenyl	Phenyl-Hexyl	NH2
Solid phase						
A: Retentivity B: Hydrophobicity C: Stereoselectivity D: Hydrogen bond property E: π-π F: Inertness						
Functional group	Octadecyl group	Octadecyl group	Octyl group	Phenyl group	Phenylhexyl group	Aminopropyl group
Particle size	2µm, 3µm, 5µm	1.9µm, 3µm, 5µm	2µm, 3µm, 5µm	2µm, 3µm, 5µm	3µm, 5µm	3µm, 5µm
Pore size	10nm	10nm	10nm	10nm	10nm	10nm
Surface area	350m²/g	350m²/g	350m²/g	350m²/g	350m²/g	350m²/g
Carbon loading	14%	13%	8%	10%	9%	7%
End cap	Yes	Yes	Yes	No	Yes	No
Recommended pH range	1-10	1-10	1-10	2-7.5	1-10	2-7.5

Fig. 3 Lineup and Features of Shim-pack GIST Series

Table 1 Analytical Conditions
(Top) Shim-pack GIST Phenyl, (Bottom) Shim-pack GIST C18

System	: Nexera™-i
Column 1	: Shim-pack GIST Phenyl (100 mm L. × 3.0 mm I.D., 2 µm)
Mobile Phase	: Water / Methanol = 80/20 (v/v)
Flow Rate	: 0.4 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 5 µL
Detection	: UV-VIS detector (Nexera-i) at 254 nm
System	: Nexera-i
Column 2	: Shim-pack GIST C18 (100 mm L. × 2.1 mm I.D., 2 µm)
Mobile Phase	: Water / Methanol = 70/30 (v/v)
Flow Rate	: 0.2 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 2.5 µL
Detection	: UV-VIS detector (Nexera-i) at 254 nm

Comparison of Organic Solvents by Using Phenyl Column

Methanol and acetonitrile are organic solvents that are frequently used as the mobile phase in RPC. Fig. 4 shows the chromatograms of standard solutions of *o*-, *m*-, and *p*-cresol (20 mg/L each) obtained by using a Shim-pack GIST Phenyl with methanol or acetonitrile as the mobile phase. Table 2 shows the analytical conditions.

Using methanol as the mobile phase facilitates the use of the π-π interaction. Acetonitrile (CH_3-C☰N) has a C-N triple bond and contains π electrons, whereas methanol (CH_3-OH) does not contain π electrons. The retentivity of methanol is greater than that of acetonitrile, as π electrons do not influence the π-π interaction between the solid phase phenyl group and the solute cresol when methanol is used.

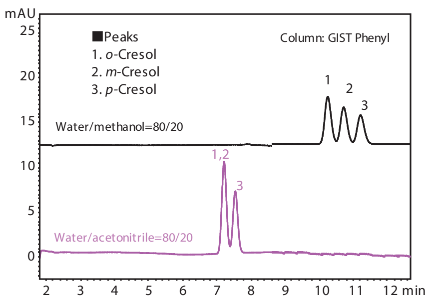


Fig. 4 Comparison of Chromatograms of Cresol Positional Isomers by Using Shim-pack GIST Phenyl (Top) Methanol, (Bottom) Acetonitrile

Table 2 Analytical Conditions (Comparison of Organic Solvents)

System	: Nexera-i
Column 1	: Shim-pack GIST Phenyl (100 mm L. ×3.0 mm I.D., 2 µm)
Mobile Phase	: Water / Acetonitrile = 80/20 (v/v), Water / Methanol = 80/20 (v/v)
Flow Rate	: 0.4 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 5 µL
Detection	: UV-VIS detector (Nexera-i) at 254 nm

Linearity of Calibration Curves

Fig. 5 shows calibration curves analyzed under the conditions of Table 1 (top). The calibration curves were prepared in the range of 5 to 20 mg/L. Satisfactory linearity with a contribution ratio r^2 = 0.999 or higher was obtained for all components.

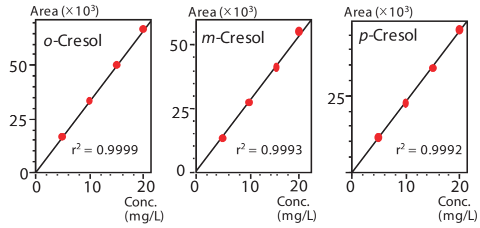


Fig. 5 Calibration Curves
Left: *o*-Cresol, Center: *m*-Cresol, Right: *p*-Cresol

Analysis of Cresol in Lignin Extracts

This section introduces an example of analysis of cresol in lignin extracts. Lignins remain as a residue component when cellulose and hemicellulose are removed from wood. Until now, lignins had been treated as a waste or used as a fuel. However, in recent years, lignophenol *1, using cresol and other phenolic compounds obtained from lignins as plant biomass, has attracted attention as a basic material (1). In the future, lignophenol is expected to be used as a substitute material for petroleum-derived plastics, which accelerate global warming. Fig. 6 shows the chromatograms obtained by analysis of lignin extracts A *2 and B *3 with standard addition of *o*-, *m*-, and *p*-cresols to the samples after 100× dilution with the mobile phase and filtration.

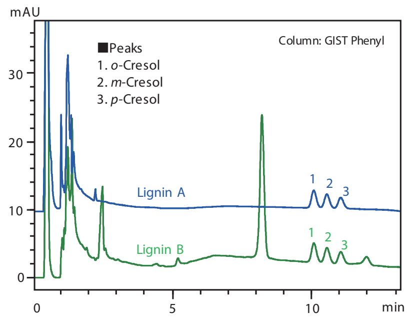


Fig. 6 Chromatograms of Lignin Extracts A and B (Standard Addition of Cresol)

Conclusion

Shim-pack GIST Phenyl displays a π-π interaction owing to its phenyl ring structure, and is an effective choice as a column when the separation performance of ODS columns is inadequate. Effective use is expected, including use as a column in method scouting for comprehensive study of separation conditions.

*1 Lignophenol: A new chemical substance developed as a bioplastic raw material in the 1990s by Prof. Emeritus Masamitsu Funaoka of Mie University.

*2 Extract obtained by treating wood from needle- and broad-leaved trees with sodium sulfite.

*3 Liquid obtained from extract in *2 by further desulfonation (partial), oxidation, hydrolysis, demethylation, and adjustment to alkalinity.

References

(1) Shin-Kobe Technical Report, No. 17 (2007-2) Effects of Molecular Structures of Lignins on the Performance of Negative Electrodes in Lead-Acid Batteries: Hitachi Chemical Co., Ltd. (Japanese)

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