

# Evaluation of Novel Cholesterol Bonded Silica Packing Material for Liquid Chromatography

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## Abstract :

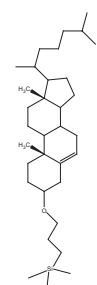
Octadecyl group bonded silica gel (C18, ODS) has been most commonly used to separate various compounds since HPLC technique was developed. However as the number of chemicals discovered and synthesized grows, there are many hard-to-analyze compounds with ODS even after optimizing the analytical conditions. Altering the selectivity of the stationary phase can result in improvements on a higher scale. We have been developing new packing materials with specific separation properties. Our latest success is 3-[(Cholesteryl)oxy]propylsilyl silica packing material. The retention properties of this new packing material were compared to ODS and C30 packing materials. The new stationary phase shows the same hydrophobicity as ODS, therefore analysis is possible under the same conditions as for ODS. To evaluate the usefulness of the new packing material, the separation factor, pattern, and the stereo selectivity arising from its rigid-structure is compared with ODS and C30.

## Background

- When you analyse unknown compounds using HPLC, the first choice would be ODS.
- The common and best method to analyse compounds which are difficult to separate with ODS is change the stationary phase.
- Changing stationary phase is not popular because it leads the difference of hydrophobicity and degree of selectivity, and the uncertainty of baseline, and so on.

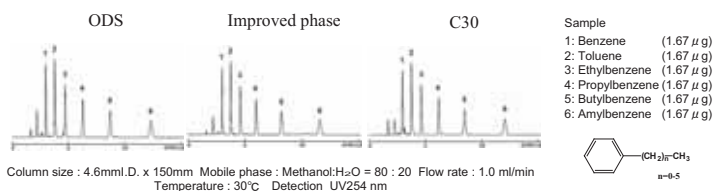
## Intention

- Show same hydrophobicity as ODS, but selectivity is a little different from ODS.
- We focus attention on the RIGID structure of cholesterol.



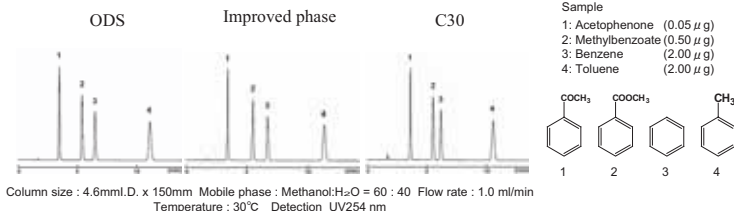
Improved type

## <Comparison of Hydrophobicity>

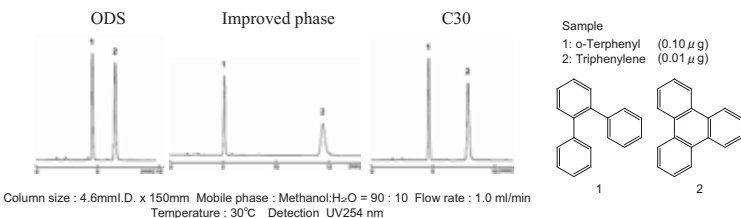


Consider the similarity of hydrophobicity, improved phase analyseable under the same condition with ODS.

## <Comparison of Selectivity for Polar Functional Group>



## <Comparison of Stereo Selectivity>



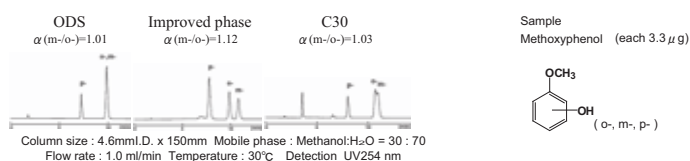
Improved phase shows the great stereo selectivity due to RIGID structure of cholesterol.

## <Comparison of factors>

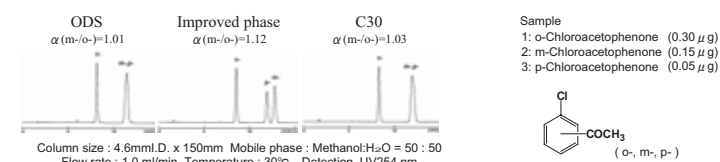
	ODS	Improved phase	C30
Hydrophobicity k' (No.6)	6.35	6.14	6.01
Selectivity for Polar Functional Group $\alpha$ (No.2/No.3)	0.77	0.76	0.85
Stereo Selectivity $\alpha$	1.31	2.24	1.62

The results from hydrophobicity and unique selectivity, we focused position isomer, geometric isomer and polyaromatic compounds.

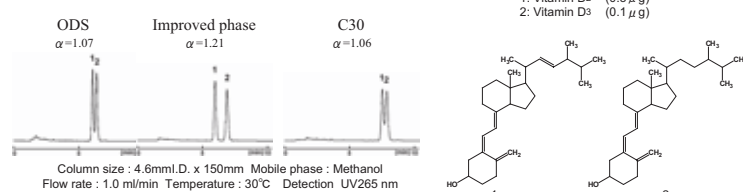
## <Methoxyphenol> (o-, m-, p- isomer)



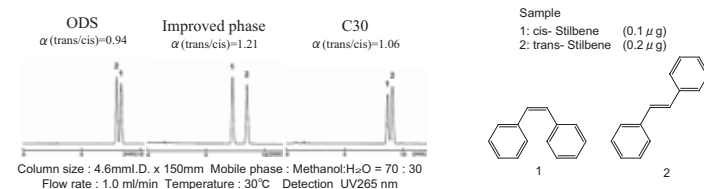
## <Chloroacetophenone> (o-, m-, p- isomer)



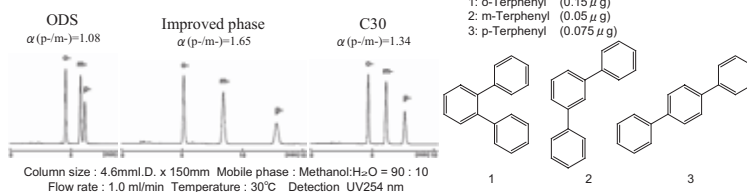
## <Vitamin D> (with or without double-bond)



## <Stilbene> (cis-, trans isomer)

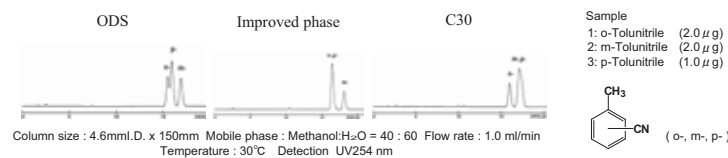


## <Terphenyl> (o-, m-, p- isomer)



As investigations proceed, we found the improved phase has a high degree of usability for isomer, double-bonded and polyaromatic compounds. In some cases, however, ODS shows the excellent result.

## <Tolunitrile>



## Conclusion :

We have been trying to develop more efficiency and performed stationary phase. Our latest improved phase is able to not only separate compounds under the same condition as for ODS, but also separate isomer, double-bonded, and polyaromatic compounds. Although ODS column provide better performance than our improved phase in some cases, the new column can be the best choice for an alternate column.