# ADVANTAGES OF A NOVEL STATIONARY PHASE USING 2.5 μM PARTICLES FOR ULTRA-FAST LIQUID CHROMATOGRAPHY.



Toshi Ono<sup>1</sup>, Jing Xu<sup>1</sup>, Tsunehisa Hirose<sup>2</sup>, Kazuhiro Kimata<sup>2</sup> <sup>1</sup>Nacalai USA, Inc., San Diego, United States, <sup>2</sup>Nacalai Tesque, Inc., Kyoto, Japan

Cholestervl Groun

# Abstract

In recent years significant attention has been attracted to Ultra-Fast Liquid Chromatography (UFLC) or the use of columns with sub-2  $\mu$ m particles for very fast and efficient chromatographic separations. The advantages of columns packed with sub-2  $\mu$ m particles used in an ultra high pressure liquid chromatography system have been well documented. Although high efficiency and fast throughput can be achieved with small particles, selectivity is still one of the most important factors for chromatographic separation and it does not depend on particle size. In order to obtain optimum resolution, different bonded phases are always desired. In this study, we demonstrate the advantages of a novel cholesteryl group bonded stationary phase using 2.5  $\mu$ m particles for UFLC. Although hydrophobic characteristics of this stationary phase are the same as those of conventional C18 columns, it shows superior selectivity for separation of structural isomers and other closely related compounds. The better separation of these compounds can be achieved with this novel 2.5  $\mu$ m column, this novel cholesteryl group bonded phase can be used with both traditional HPLC systems and new UHPLC systems.

# Introduction

UFLC with sub-2  $\mu$ m particles has become a powerful tool for very fast and efficient separation. However, conventional phase chemistries may not provide optimum separation for some compounds with structural similarities. This study demonstrated the characteristics and advantages of a novel cholesteryl group bonded stationary phase, compared to the most commonly used C18 stationary phase.

# Material characteristics

Silica Gel	High Purity Spherical Silica	
Stationary Phase	Cholesteryl group	
Average Particle Size	2.5 μm	
Average Pore Size	130 Å	
Surface Area	330 m²/g	
Carbon content	21%	

#### Stationary phase characteristics: Planarity selectivity



## Ultra-Fast Liquid Chromatography: 2.5 µm vs. 1.7 µm

#### Figure 4 : Separation of Saikosaponin mixture

Cholesteryl stationary phase with 2.5  $\mu$ m particles shows superior selectivity and significantly lower back pressure than those of 1.7  $\mu$ m C18 stationary phase.



# Ultra-Fast Liquid Chromatography : 2.5 µm vs. 1.7 µm



## Stationary phase characteristics: Hydrophobicity

#### Figure 1

Alkylbenzenes are used to determine hydrophobic interaction. Cholesteryl stationary phase and conventional ODS show the same hydrophobic interaction.



#### Stationary phase characteristics: Analog selectivity



## Ultra-Fast Liquid Chromatography : 2.5 µm vs. 2.2 µm

#### Figure 5 : Separation of Catechins

Cholesteryl stationary phase shows better selectivity than ODS stationary phase



# Conclusions

- COSMOSIL Cholester shows the same hydrophobicity as conventional C18 columns, which makes it easy to optimize mobile phase conditions.
- Because of the rigid structure of cholesteryl group, COSMOSIL Cholester shows greater planarity selectivity and better resolution of geometric isomers.
- COSMOSIL Cholester with 2.5 um particles shows lower back pressure than a C18 stationary phase with 1.7 um particles under the same flow rate.
- ≻For UFLC, COSMOSIL Cholester with 2.5 µm particles offers superior separation with a short analysis time as compared to a C18 stationary phase with 1.7 µm particles.