Advantages of ultra-fast liquid chromatography using 2 μm packing materials

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1. Introduction

Advantages of ultra-fast LC are known as shorter analysis time, higher resolution and higher productivity than conventional LC. But in almost analyses, it is necessary to use specific LC instrument due to high back pressure produced by sub-2 μm particles. And another demerit, commercially available sub-2 μm columns show different selectivity compared to existing conventional columns.

We developed new 2 μm particle columns called YMC-UltraHT series, which designed for highly compromised advantages and disadvantages of current commercial columns for ultra-fast LC. Our 2 μm columns show almost same efficiency of sub-2 μm columns with about 40 % lower back-pressure.

This low column pressure allows it to use ordinary LC systems with maximum pressure of 6,000 psi even at a high flow rate. Two types of ODS phases are available, which have same selectivity compared to 3 μm, 5 μm *Pro* C18 and Hydrosphere C18, respectively. For that reason, it is easy to scale down to 2 μm columns from conventional particle size without changing eluent condition. In this poster, we will show advantages of YMC-UltraHT columns and usage under ultra-fast LC conditions.

2. YMC designs

High column efficiency with minimum column pressure

- Functionalized ultra-pure 2.0 μm silica gel with 120 Å pore size.
- Designed for lower pressure but better performance than sub-2 μm particles.
- Instruments are not only specific ultra-fast LC but ordinary LC available.

Easy scalability of separation

- Two types of bonded phase with different selectivities; *Pro* C18 as standard ODS and Hydrosphere C18 for highly-polar compounds.
- Identical selectivity as 3 μm or 5 μm conventional *Pro* series columns with same bonded phase.
- Applicable for various compounds such as pharmaceuticals, foods and natural products.

Column type

Particles	: Spherical ultra-pure silica 2 μm particle size, 120 Å pore size
Funcitonal group	: *Pro* C18 (standard ODS) Hydrosphere C18 (sperior selectivity of highly polar compounds)
Column size	: Φ2 mm X 30, 50, 75, 100 mm Φ3 mm X 50, 75, 100 mm
Available pH	: pH 2-8

3. Advantage of YMC-UltraHT series

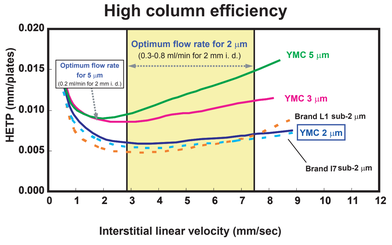


Fig.1 Van Deermter curves

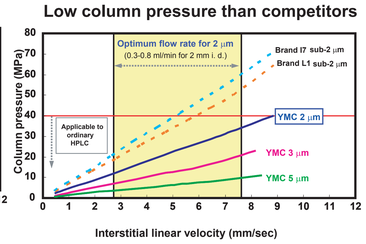
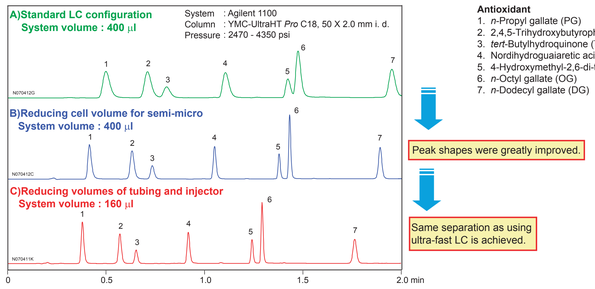


Fig.2 Liner velocity versus column pressure

YMC 2 μm column shows almost same efficiency to competitors' sub-2 μm in 0.3 - 0.8 ml/min for 2 mm i. d. of flow rate (Fig.1). On the other hand, YMC 2 μm shows apparently lower column pressure than competitors' (Fig.2). Thus, it concludes YMC 2 μm column shows same efficiency in 40% lower column pressure than competitors'.

Column : 50 x 2.0 or 2.1 mm i. d.
Eluent : acetonitrile / water (60/40)
Temperature : 25°C
Detection : UV at 254 nm
Sample : Butyl benzoate

Applicable to ordinary LC systems

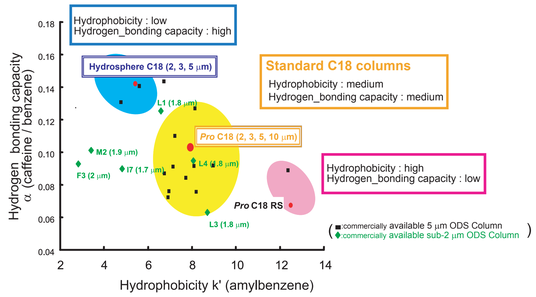


Eluent : A) water / TFA (100/0.1)
B) acetonitrile / methanol / TFA (75/25/0.1)
45-70%B (0-1 min), 70-95%B (1-1.33 min), 95%B (1.33- 2 min)
Flow rate : 0.6 ml/min
Temperature : 30°C
Detection : UV at 280 nm
Injection : 1 μl

YMC 2 μm enables ultra-fast analysis with low column pressure compatible with ordinary LC systems. As shown in Figs.3, by minimizing the dead volume of our existing HPLC (such as cell, tubing, and injector's volume), the peak shapes are improved and seven antioxidants can be separated completely within 2 minutes.

Figs.3 Optimization of ordinary HPLC for ultra-fast analysis

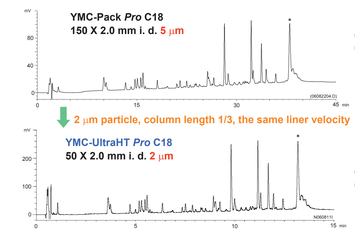
Two types of C18 bonded-phase with different selectivities



2 μm UltraHT series have two types of C18 bonded-phase with different selectivity, which is the same as 3 μm/5 μm Pro C18 and Hydrosphere C18, respectively (Fig.4). For that reason, it is easy to scale down to 2 μm column from conventional particle size.

Fig.4 Comparison of selectivity balance between K' and α

Identical selectivity within same bonded-phase

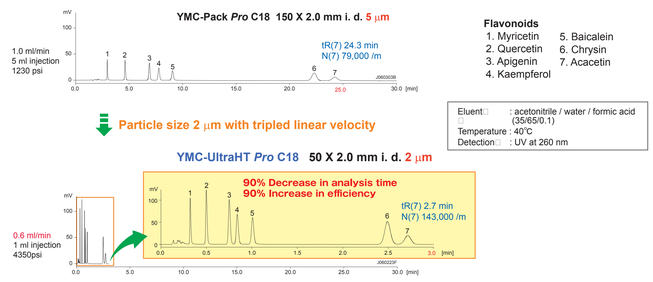


Eluent : A) acetonitrile / water / TFA (10/90/0.1)
B) acetonitrile / water / TFA (35/65/0.1)
Flow rate : 0.2 ml/min
Temperature : 37°C
Detection : UV at 220 nm
Sample : Tryptic digest of Cytochrome *c*
*undigested Cytochrome *c*

2 μm UltraHT *Pro* C18 shows same selectively as 5 μm *Pro* C18 even in the complex and demanding separation such as peptide mapping (Figs.5).
YMC 2 μm gives excellent reproducibility between different particle sizes.

Figs.5 Selectivity comparison of different particle size

Easy method transfer from conventional LC to Ultra-fast LC

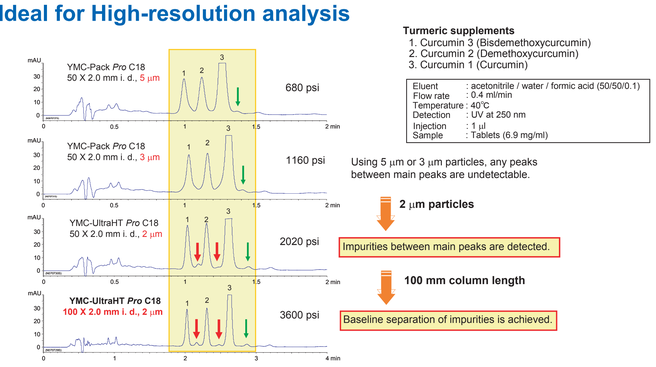


Eluent : acetonitrile / water / formic acid (35/65/0.1)
Temperature : 40°C
Detection : UV at 260 nm

Figs.6 shows method transfer from conventional LC with 5 μm 150 X 4.6 mm i. d. to ultra-fast LC with 2 μm 50 X 2.0 mm i. d.. It takes 30 min with conventional column, while YMC 2 μm gives a 90% reduction of analysis time and a 90% increase of column efficiency without changing eluent condition and separation pattern.

Figs.6 Method transfer from conventional column to YMC 2 μm column

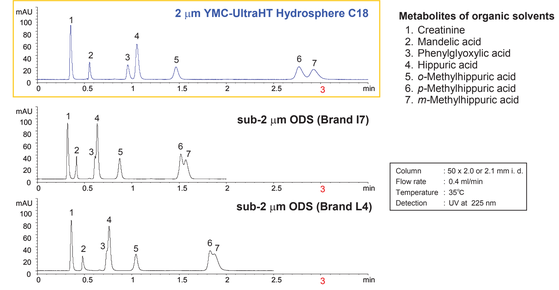
Ideal for High-resolution analysis



Figs.7 Optimization of analytical method

Using conventional column, any peaks between each of main components are undetected. However, by scaling down of particle size to 2 μm, some impurities are detected. Furthermore, the baseline separation is achieved by changing column length to 100 mm (Figs.7).
YMC 2 μm is applicable for high-throughput analysis such as pharmaceuticals agricultural chemicals and foods. Especially, column length 100 mm is useful for high-resolution analysis.

Hydrosphere C18 gives superior selectivity of highly polar compounds



Column : 50 x 2.0 or 2.1 mm i. d.
Flow rate : 0.4 ml/min
Temperature : 35°C
Detection : UV at 225 nm

Figs.8 Comparison of selectivity for highly polar compounds

2 μm Hydrosphere C18 shows superior selectivity and resolution of polar metabolites of organic solvents in a highly aqueous mobile phase (Figs.8). The other sub-2 μm columns can not obtain favorable retention and resolution. Selectivity is one of the most important factor for developing faster analysis time and more efficient method.

4. Conclusions

- YMC 2μm achieved about 40% lower back pressure with same efficiency as commercial sub-2 μm columns, due to functionalized ultra-pure 2.0 μm silica gel optimized packing.
- Two types of ODS phase are available; *Pro* C18 and Hydrosphere C18 which have same selectivity with conventional 3 μm, 5 μm *Pro* series columns.
- Simple method transfer between conventional LC and ultra-fast LC is available without changing eluent conditions.