Ultra high-throughput analyses of polar compounds with newly developed 2 μm YMC-UltraHT C18 column

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YMC SEPARATION TECHNOLOGY

1. Introduction

Recently, ultra-fast LC has been widely applied to analysis of various fields such as pharmaceuticals, foods, and environmental. Although commercial columns packed with sub-2 μm or 2 μm particles give improved efficiency and reduced analysis time, acceptable retention or separation has not been often achieved in many hydrophilic compounds such as metabolites or natural compounds.

To solve these problems, we have lined up a new category of products, YMC-UltraHT Hydrosphere C18, into our 2 μm columns for ultra-fast LC. The balanced hydrophilic/hydrophobic nature of this packing material provides strong retention and superior selectivity of highly polar compounds. Also this new column maintains all the advantages of 2 μm YMC-UltraHT *Pro* C18 column. Our 2 μm columns show almost same efficiency of sub-2 μm columns with about 40 % lower back-pressure and they can be used with ordinary LC systems.

In this poster, we will show some example cases of fast and efficient separation of pharmaceuticals and metabolites containing polar compounds. We will also compare the retention and elution achieved with Hydrosphere C18 and those achieved with other C18 columns for ultra-fast LC.

2. Features of YMC-UltraHT columns

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

■ Identical selectivity as 3 μm or 5 μm conventional *Pro* series columns within same bonded phase.

■ Applicable for various compounds such as pharmaceuticals, foods and natural products.

■ Simple method transfer applicable without changing eluent conditions.

3. New UltraHT column for polar compounds

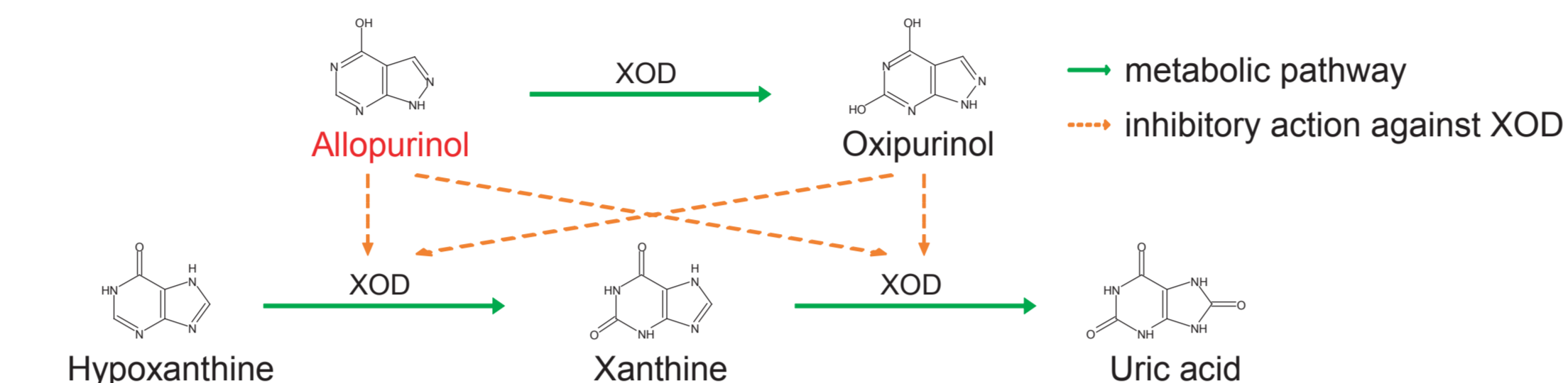### YMC-UltraHT Hydrosphere C18

■ Designed for strong retention and superior resolution selectivity of polar compounds.

■ Excellent reproducibility of retention time under 100 % aqueous condition.

■ Unique selectivity that differs from standard ODS, *Pro* C18.

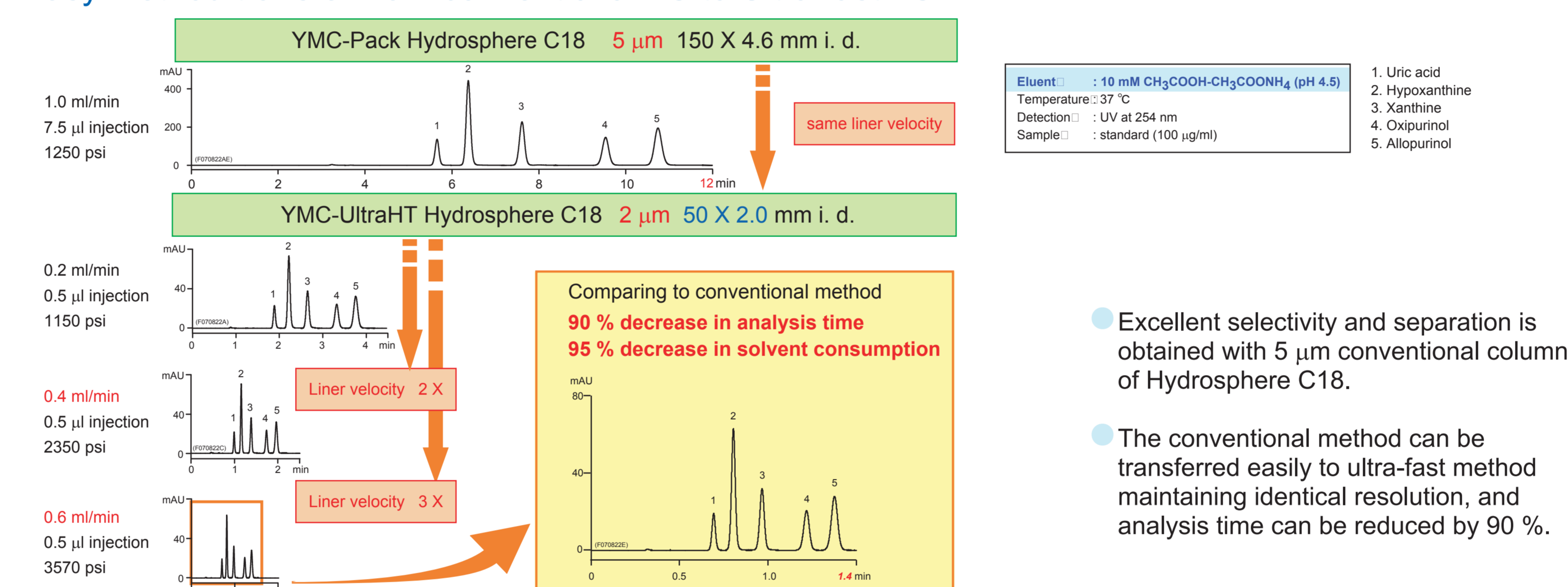|	Hydrosphere C18	*Pro* C18
feature	for polar compounds	standard type ODS
particle size (μm)	2, 3, 5	2, 3, 5, 10
carbon contents (%)	12 %	16 %
bonded phase	monomeric	monomeric
end-capping	completely end-capped	completely end-capped

4. Fast and efficient separation of polar compounds on YMC-UltraHT Hydrosphere C18

Ultra-fast analysis under 100 % aqueous condition

Xanthine oxidase (XOD) inhibitor and related metabolites

— metabolic pathway
⋯ inhibitory action against XOD

Allopurinol → Oxipurinol
Hypoxanthine → Xanthine → Uric acid

● Allopurinol is a xanthine oxidase inhibitor and widely used in the treatment of hyperuricemia and gout.

● Allopurinol and its related metabolites are highly polar compounds with similar structure.

Easy method transfer from conventional LC to Ultra-fast LC

YMC-Pack Hydrosphere C18 5 μm 150 X 4.6 mm i. d.
1.0 ml/min, 7.5 μl injection, 1250 psi

YMC-UltraHT Hydrosphere C18 2 μm 50 X 2.0 mm i. d.
0.2 ml/min, 0.5 μl injection, 1150 psi
0.4 ml/min, 0.5 μl injection, 2350 psi — Liner velocity 2 X
0.6 ml/min, 0.5 μl injection, 3570 psi — Liner velocity 3 X

same liner velocity

Eluent : 10 mM CH₃COOH-CH₃COONH₄ (pH 4.5)
Temperature : 37 ℃
Detection : UV at 254 nm
Sample : standard (100 μg/ml)

1. Uric acid
2. Hypoxanthine
3. Xanthine
4. Oxipurinol
5. Allopurinol

Comparing to conventional method
90 % decrease in analysis time
95 % decrease in solvent consumption

● Excellent selectivity and separation is obtained with 5 μm conventional column of Hydrosphere C18.

● The conventional method can be transferred easily to ultra-fast method maintaining identical resolution, and analysis time can be reduced by 90 %.
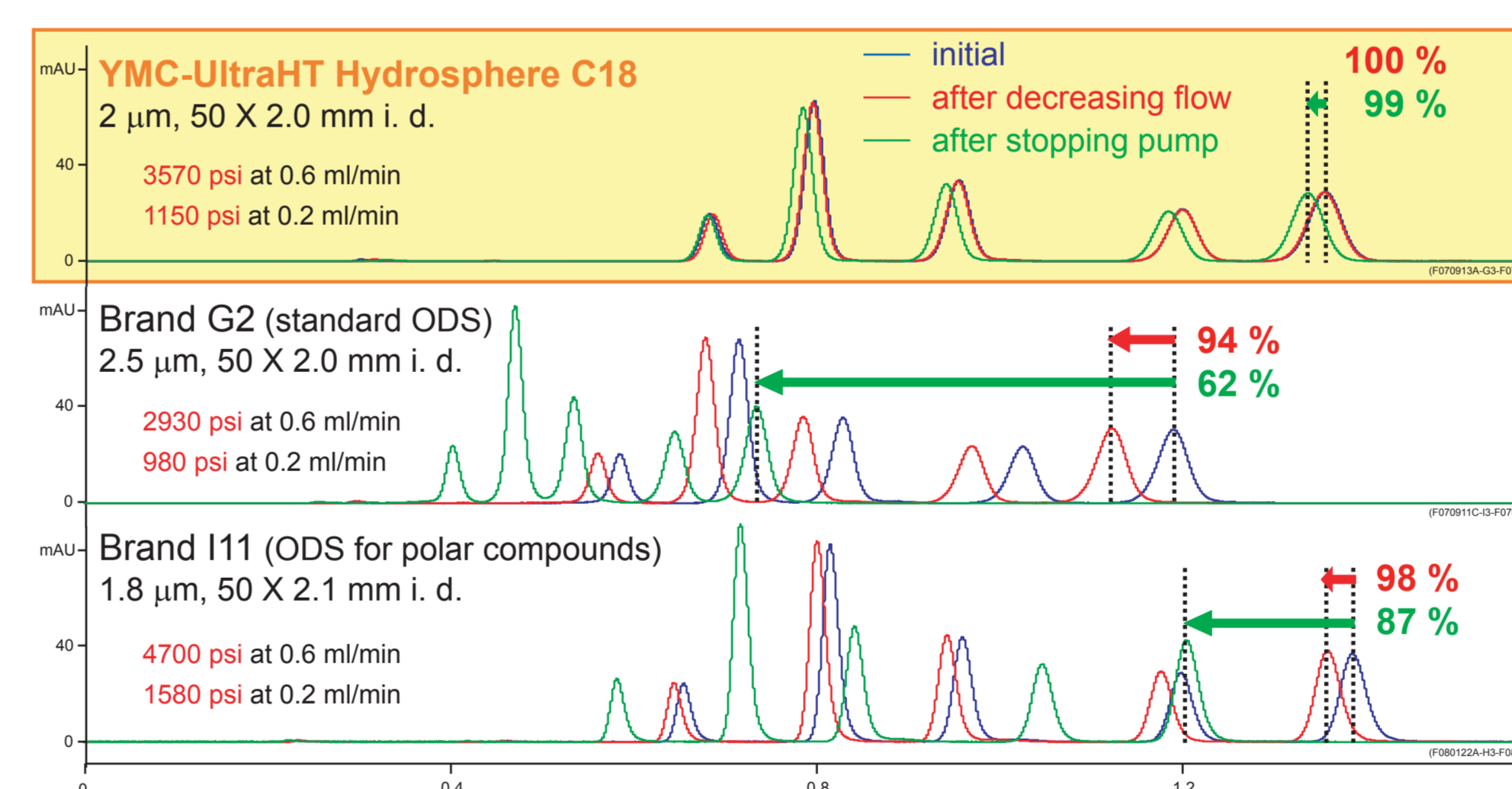
Comparison of retention stability in 100% aqueous mobile phase

Evaluation method

· Analytical condition
same as the optimized method in above figure

· Test procedure
analysis at 0.6 ml/min
(initial : drawn in blue)
↓
decrease to 0.2 ml/min and flow continuously for 30 min
(with lower pressure)
↓
analysis at a 0.6 ml/min
(after decreasing flow: drawn in red)
↓
stop the pump for 30 min
(with no pressure)
↓
analysis at 0.6 ml/min
(after stopping pump: drawn in green)

YMC-UltraHT Hydrosphere C18
2 μm, 50 X 2.0 mm i. d.
3570 psi at 0.6 ml/min
1150 psi at 0.2 ml/min
— initial 100 %
— after decreasing flow 99 %
— after stopping pump

Brand G2 (standard ODS)
2.5 μm, 50 X 2.0 mm i. d.
2930 psi at 0.6 ml/min
980 psi at 0.2 ml/min
94 %
62 %

Brand I11 (ODS for polar compounds)
1.8 μm, 50 X 2.1 mm i. d.
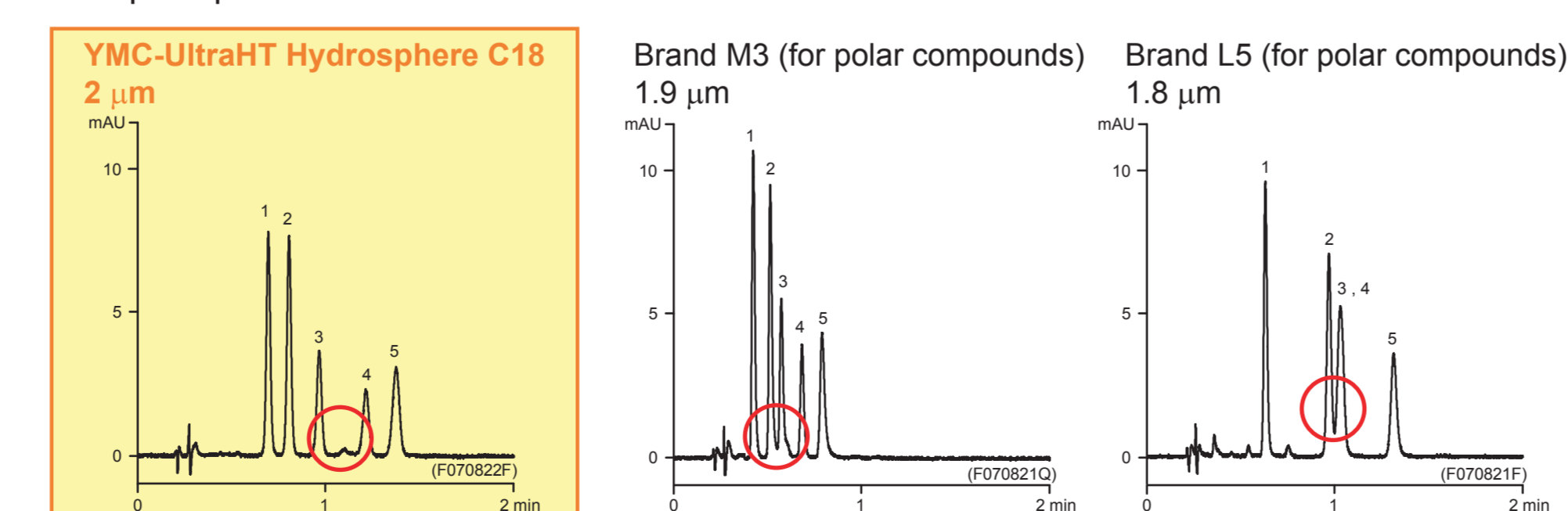4700 psi at 0.6 ml/min
1580 psi at 0.2 ml/min
98 %
87 %

● In both cases of decreasing flow rate and stopping the pump, the considerable loss of retention is observed with Brand G2 (standard ODS) and Brand I11 (ODS recommended to use in 100% aqueous mobile phase). YMC-UltraHT Hydrosphere C18 shows excellent stability and reproducibility.

● It is important to choose appropriate column for the reproducible analysis in a highly aqueous mobile phase, because 0.2 ml/min is common flow rate for 2.0 or 2.1 mm i. d. column.
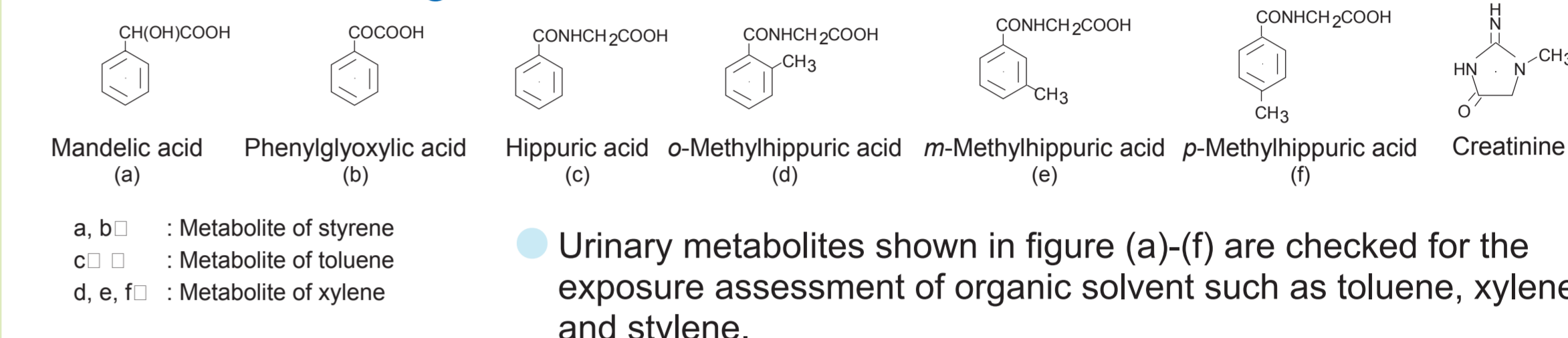
Superior selectivity comparing to commercial sub-2 μm columns for polar compounds
sample: spiked human serum

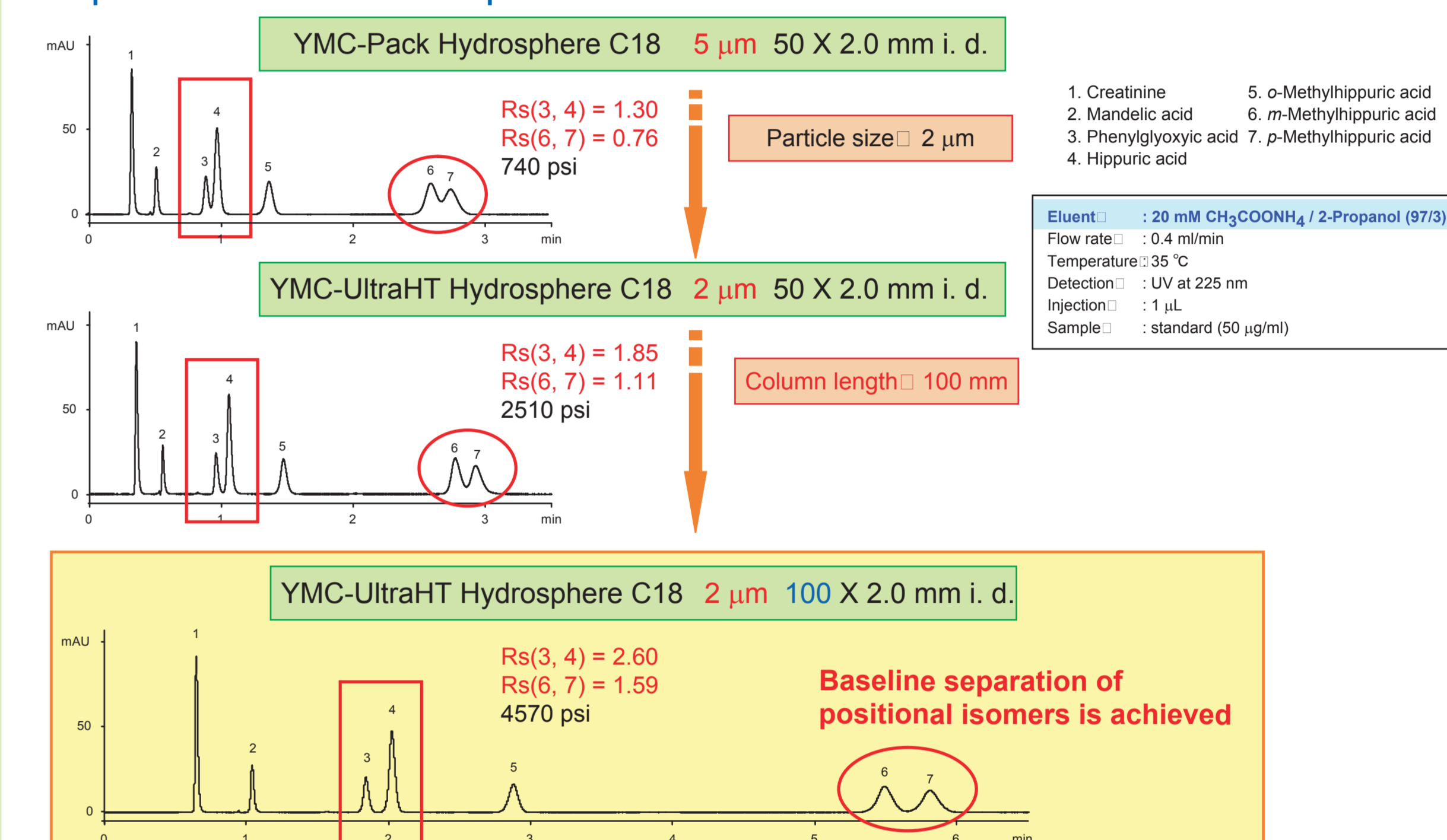YMC-UltraHT Hydrosphere C18 2 μm

Brand M3 (for polar compounds) 1.9 μm

Brand L5 (for polar compounds) 1.8 μm

● YMC-UltraHT Hydrosphere C18 provides superior selectivity and resolution than the other sub-2 μm columns designed for polar compounds.

● Brand M3 and L5 cannot achieve favorable separation, although they give good results in our evaluation of retention stability.

Ultra-high resolution of polar metabolites

Metabolites of organic solvents in urine

Mandelic acid (a), Phenylglyoxylic acid (b), Hippuric acid (c), *o*-Methylhippuric acid (d), *m*-Methylhippuric acid (e), *p*-Methylhippuric acid (f), Creatinine

a, b : Metabolite of styrene
c : Metabolite of toluene
d, e, f : Metabolite of xylene

● Urinary metabolites shown in figure (a)-(f) are checked for the exposure assessment of organic solvent such as toluene, xylene and stylene.

Improved resolution of positional isomers

YMC-Pack Hydrosphere C18 5 μm 50 X 2.0 mm i. d.
Rs(3, 4) = 1.30
Rs(6, 7) = 0.76
740 psi
Particle size 2 μm

YMC-UltraHT Hydrosphere C18 2 μm 50 X 2.0 mm i. d.
Rs(3, 4) = 1.85
Rs(6, 7) = 1.11
2510 psi
Column length 100 mm

YMC-UltraHT Hydrosphere C18 2 μm 100 X 2.0 mm i. d.
Rs(3, 4) = 2.60
Rs(6, 7) = 1.59
4570 psi
Baseline separation of positional isomers is achieved

1. Creatinine
2. Mandelic acid
3. Phenylglyoxic acid
4. Hippuric acid
5. *o*-Methylhippuric acid
6. *m*-Methylhippuric acid
7. *p*-Methylhippuric acid

Eluent : 20 mM CH₃COONH₄ / 2-Propanol (97/3)
Flow rate : 0.4 ml/min
Temperature : 35 ℃
Detection : UV at 225 nm
Injection : 1 μL
Sample : standard (50 μg/ml)

● By changing particle size from 5 μm to 2 μm, the resolution between each peak is significantly improved.

● Furthermore, the 100 mm length column with higher efficiency can achieve baseline separation of *p*- and *m*-isomer of methylhippuric acid (peak 6 and peak 7) which are hardly separable with conventional reversed-phase columns.

Analysis of human urine sample with 2 μm 100 X 2.0 mm i.d. column

Spiked blank urine (50 μg/ml)
Blank urine (diluted 10 times with water)

● The excellent separation is obtained for all detected peaks including impurities which often affect the quantitative determination of target compounds in analysis of biological samples.
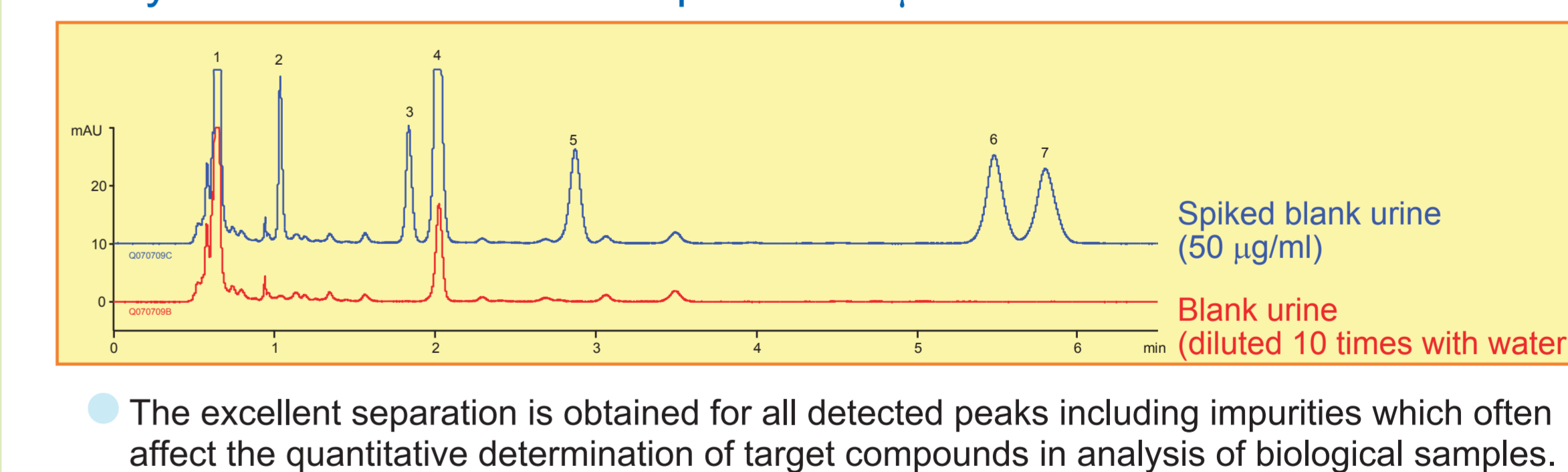
5. Conclusions

■ YMC-UltraHT Hydrosphere C18 shows same selectivity as existing 3 μm and 5 μm Hydrosphere C18, so easy method transfer can be achieved between conventional LC and Ultra-fast LC without changing eluent condition.

■ YMC-UltraHT Hydrosphere C18 provides superior resolution and excellent retention reproducibility of highly polar compounds even in 100 % aqueous mobile phase.

■ The combination of high efficiency and unique selectivity of YMC-UltraHT Hydrosphere C18 enables the high-throughput analyses of various pharmaceuticals, foods and natural products containing polar compounds.