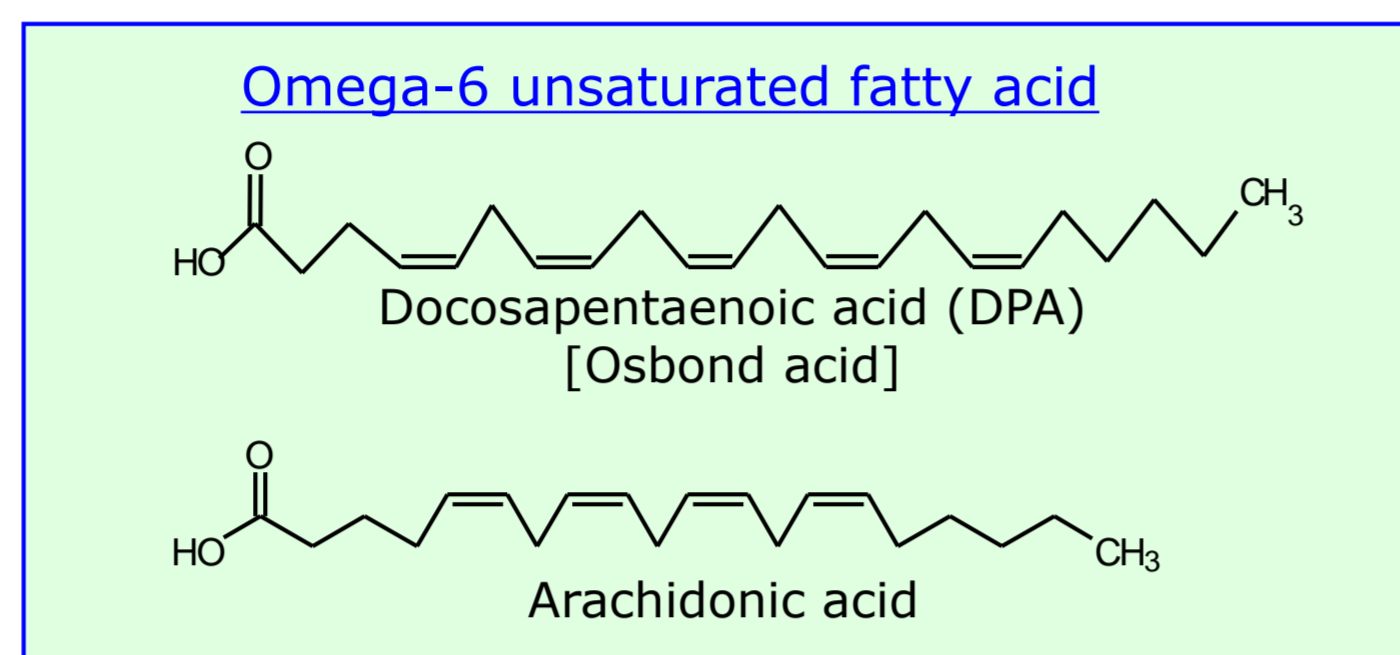
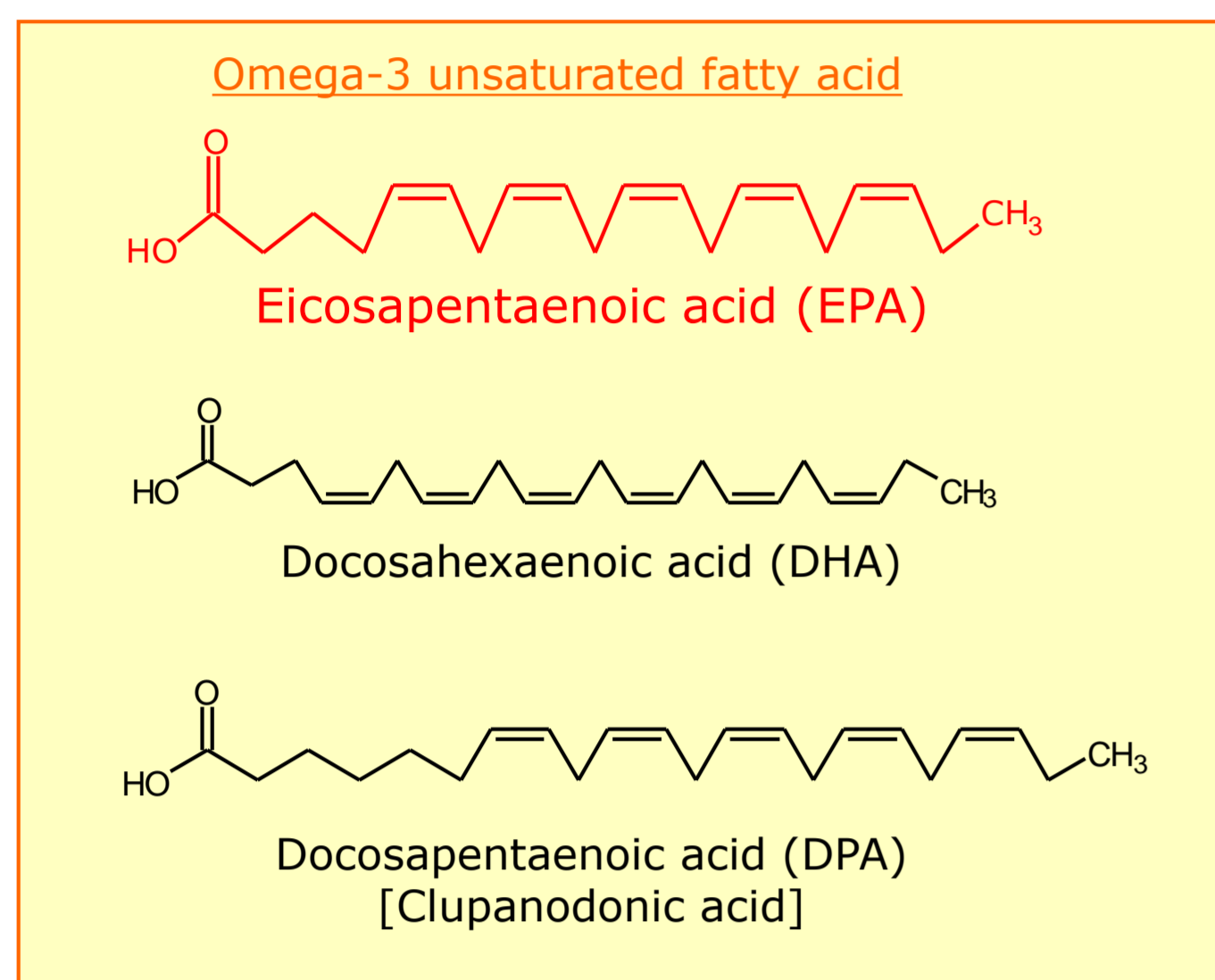


Introduction

Polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are known to have anti-atherogenic and anti-hyperlipidemic effects. Recently, the market of PUFAs has expanded rapidly, especially the demand for high purity EPA ethyl ester (EPA-EE; > 95%) as an active pharmaceutical ingredient. For purification of those PUFAs with high purity, liquid chromatography methods are widely used because they are considered the most efficient and reliable methods. Therefore a large-scale industrial HPLC process attracts considerable interest for the purification of EPA-EE.

In order to meet such a demand, we have developed a reversed phase packing material, named YMC-Omega, designed for highly efficient purification of EPA-EE. By using a combination of this novel chromatography resin and a large scale dynamic axial compression column, we have successfully developed a highly efficient and highly productive method for purification of EPA-EE. In this poster, we will describe the YMC-Omega packing material and the method development for purification of EPA-EE using YMC-Omega.

Structure of PUFAs



- Traditionally, fish oil has been used for source of PUFAs. Recently, micro-organisms and plants are proposed as sources of PUFAs.
- Impurity profile of PUFAs should vary depending on sources. Therefore, HPLC is needed for purification of PUFAs.
- A special stationary phase which could recognize small difference of PUFAs and their impurities is required for high loadability and high yield purification.

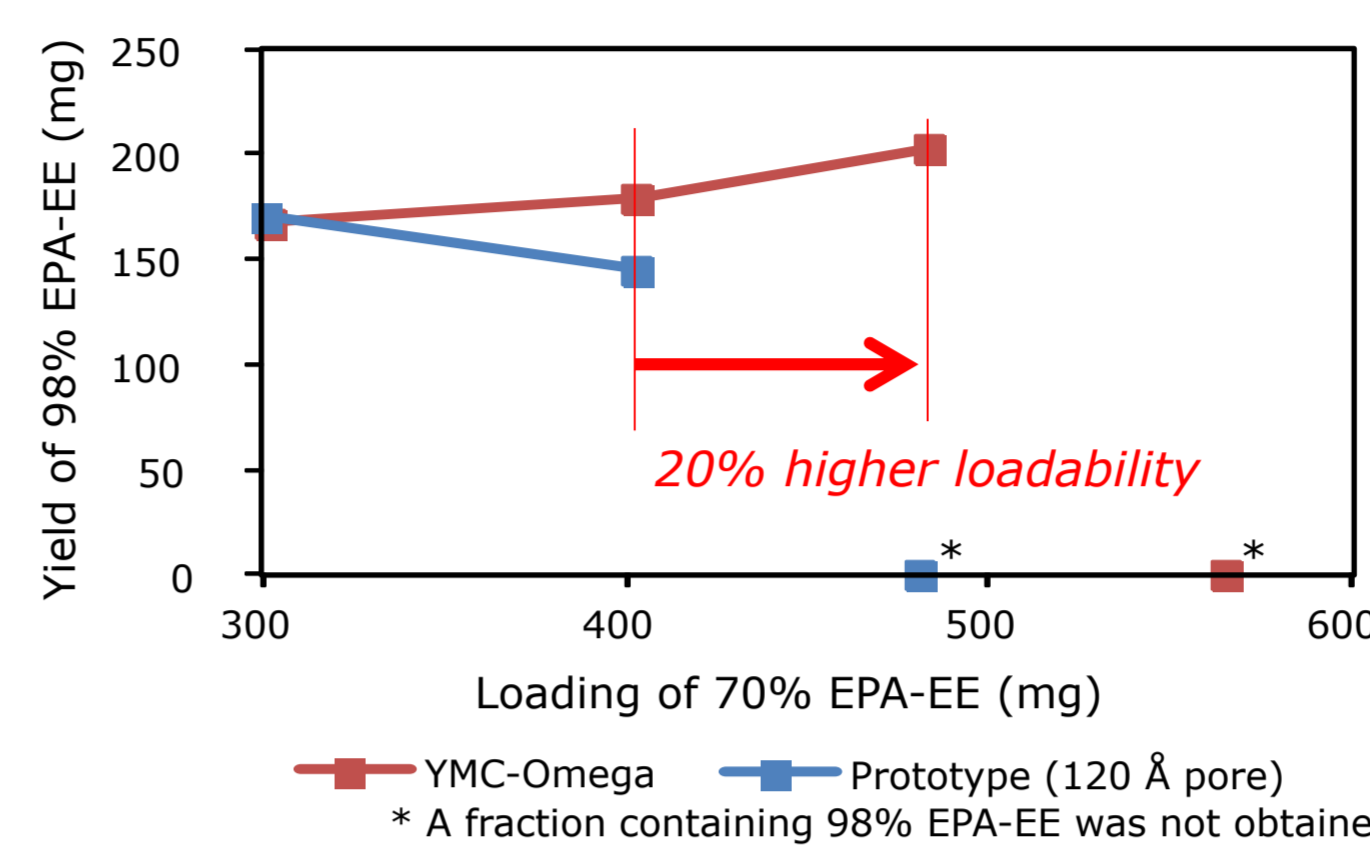
Features and Advantages of YMC-Omega

By optimizing particle characteristics and bonding, YMC-Omega shows ideal loadability and separation characteristics on EPA-EE purification under 100% methanol eluent condition which is commonly used. YMC-Omega is designed to maximize the productivity of EPA-EE.

Specifications

Base material	Porous silica
Particle size (μm)	10, 20, 50
Carbon content	15%

Optimal Pore Size



HPLC Conditions

Column	: 10 μm, 250 X 10 mm i. d.
Eluent	: methanol
Flow rate	: 2.0 ml/min
Temperature	: 25°C
Detection	: UV at 210 nm
Sample	: 70% EPA-EE (400 mg/ml)

When increasing loading, there is a certain loading of which a fraction with a desired purity (for this case, 98% EPA-EE) cannot be obtained. The threshold loading varies depending on design of packing material and separation conditions.

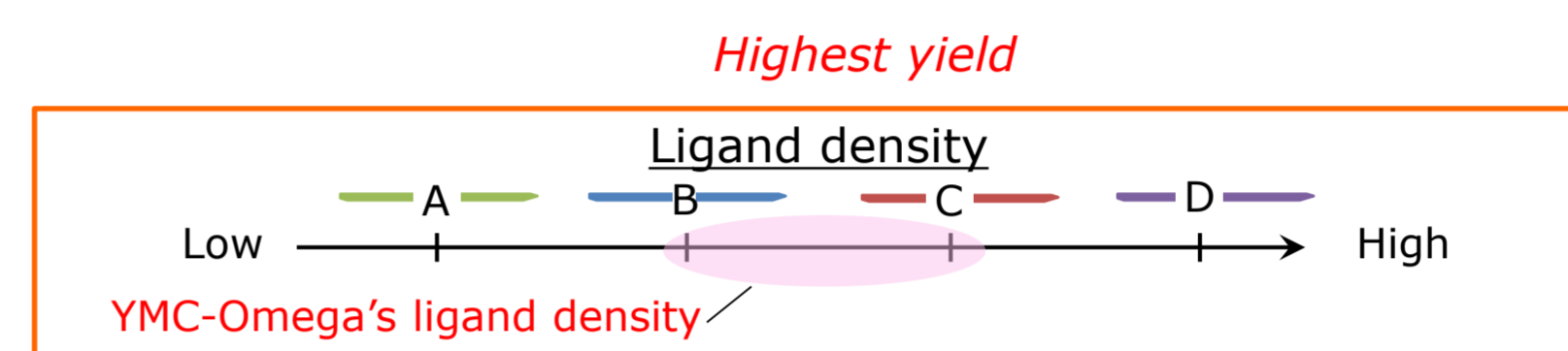
Particle pore size is one of the important factors on loadability and selectivity on EPA-EE purification. YMC-Omega with proprietary pore size could achieve 20% higher loadability than a packing material based on silica gel with 120 Å pore.

Optimal Ligand Density

Ligand density	A	B	C	D
Yield of 98% EPA-EE (mg)	172	198	207	152

HPLC Conditions

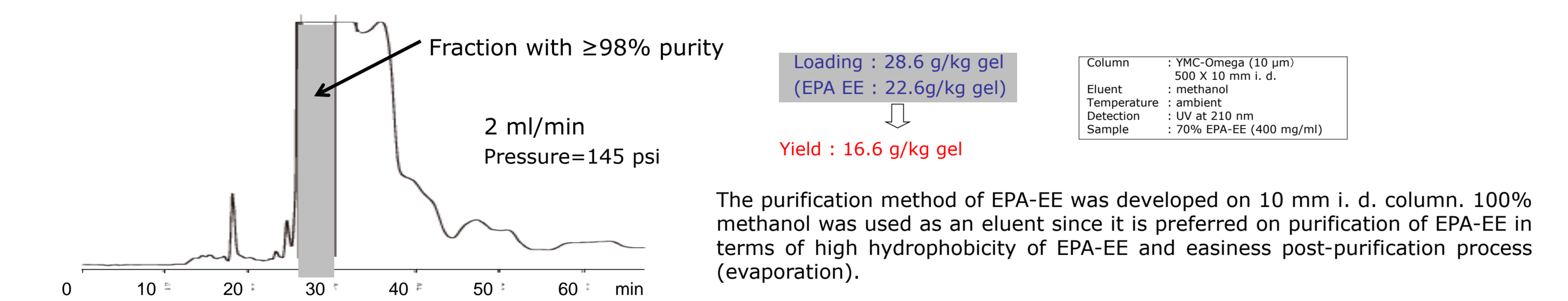
Column	: 10 μm, 250 X 10 mm i. d.
Eluent	: methanol
Flow rate	: 2.0 ml/min
Temperature	: 25°C
Detection	: UV at 210 nm
Loading	: 480 mg of 70% EPA-EE



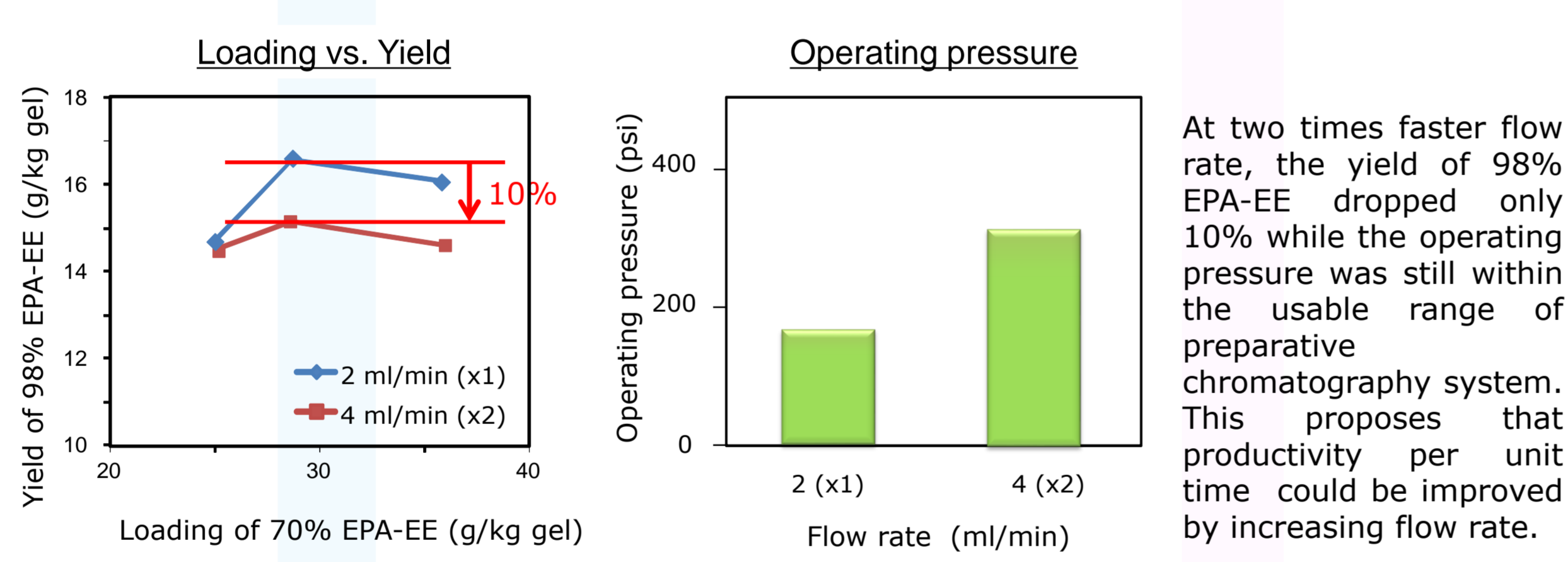
Impact of ligand density on loadability is estimated by preparing packing materials with various ligand densities. As shown in the figure, a certain range of ligand density had highest loadability. In order to secure high loadability and high productivity, the ligand density of YMC-Omega is strictly controlled.

Production of EPA-EE purification with YMC-Omega

Method development and optimization with 10 mm i. d. column

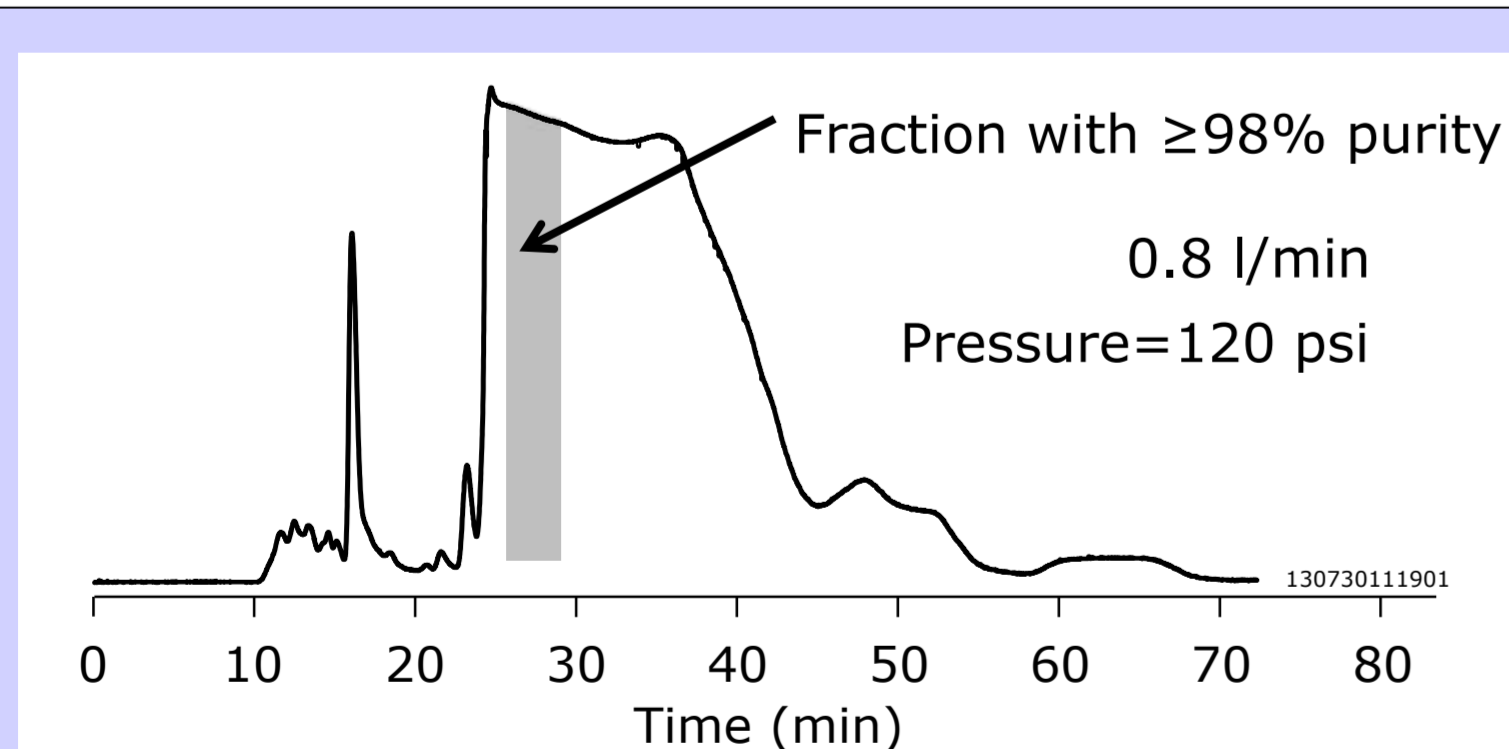


Impact of flow rate on productivity and operating pressure

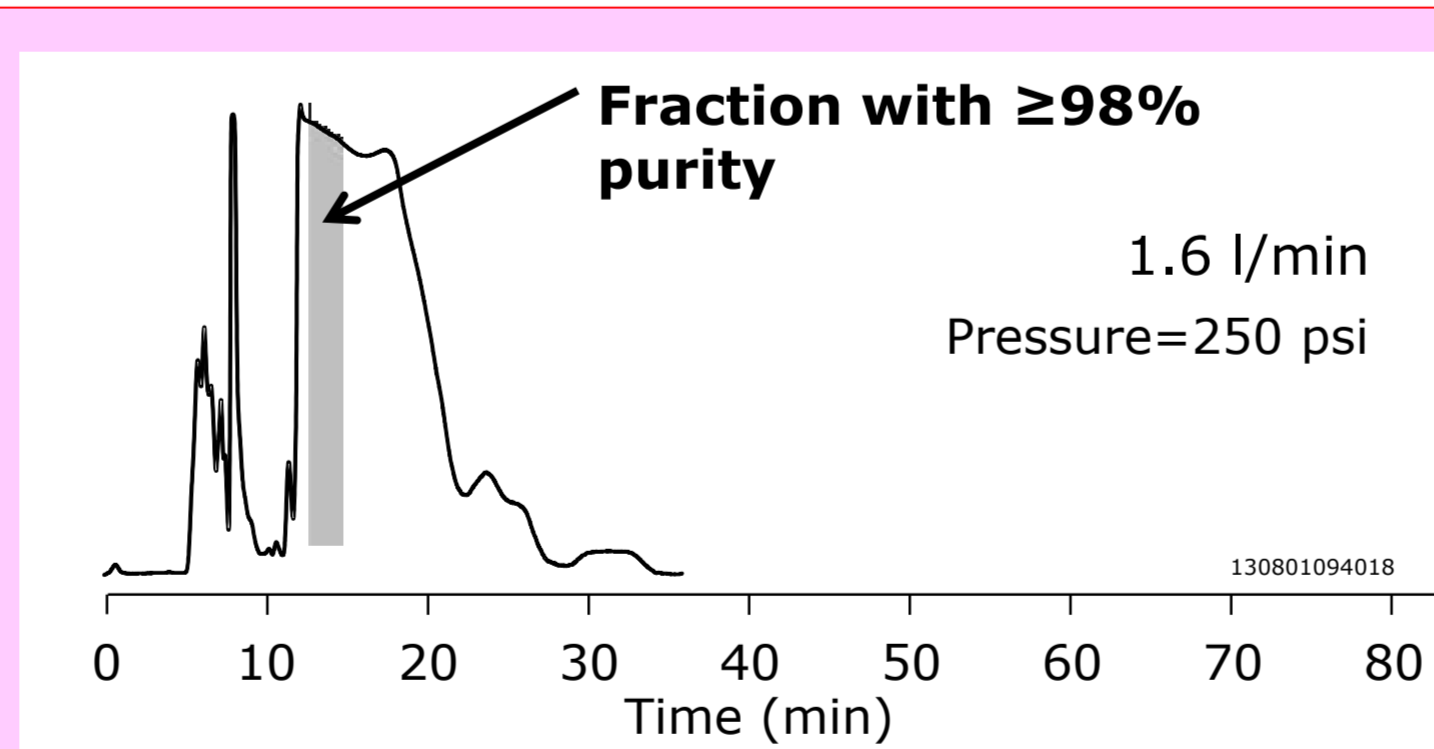


Scaling up to 500 X 200 mm i. d.

Linear scaling up (x1)

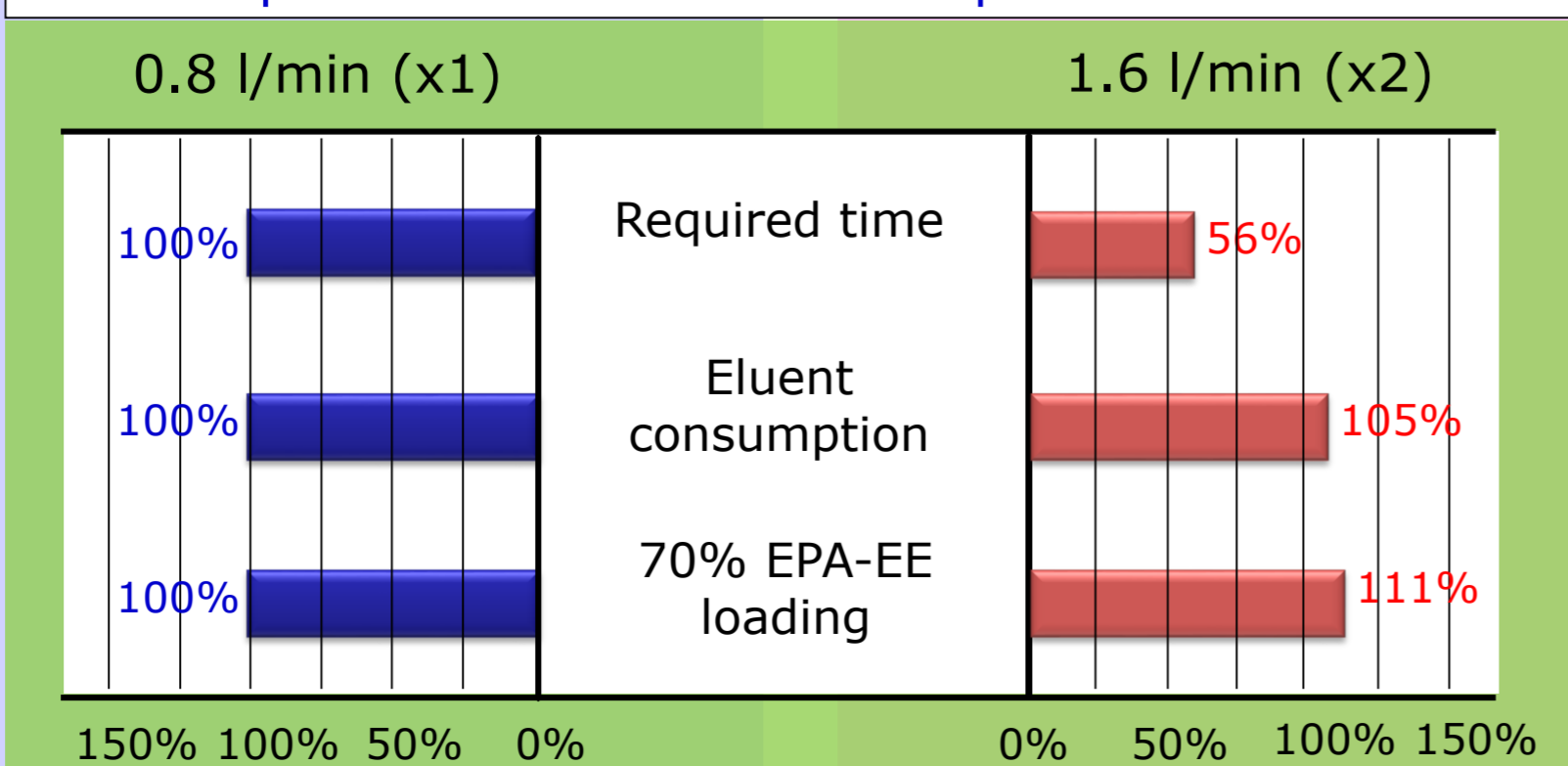


Scaling up at two times faster flow rate (x2)



0.8 l/min (x1)	Flow rate	1.6 l/min (x2)
60 min	Cycle time	30 min
1 run	Runs /hour	2 runs
30.1 g/kg gel (23.8 g/kg gel)	Loading (EPA-EE)	30.1 g/kg gel (23.8 g/kg gel)
71.8%	Theoretical yield	64.7%
17.1 g/kg gel	Productivity / hour	30.8 g/kg gel

Comparison of required time, eluent consumption and raw material per unit amount of EPA-EE production

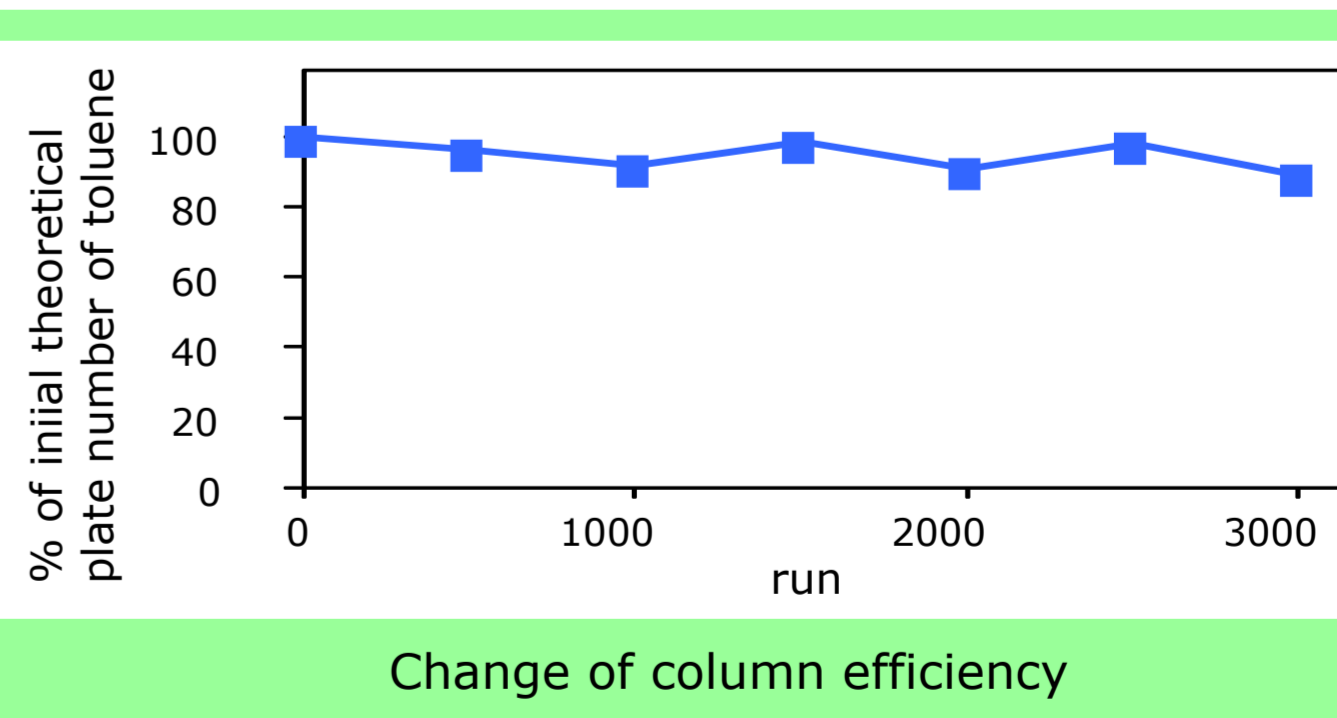
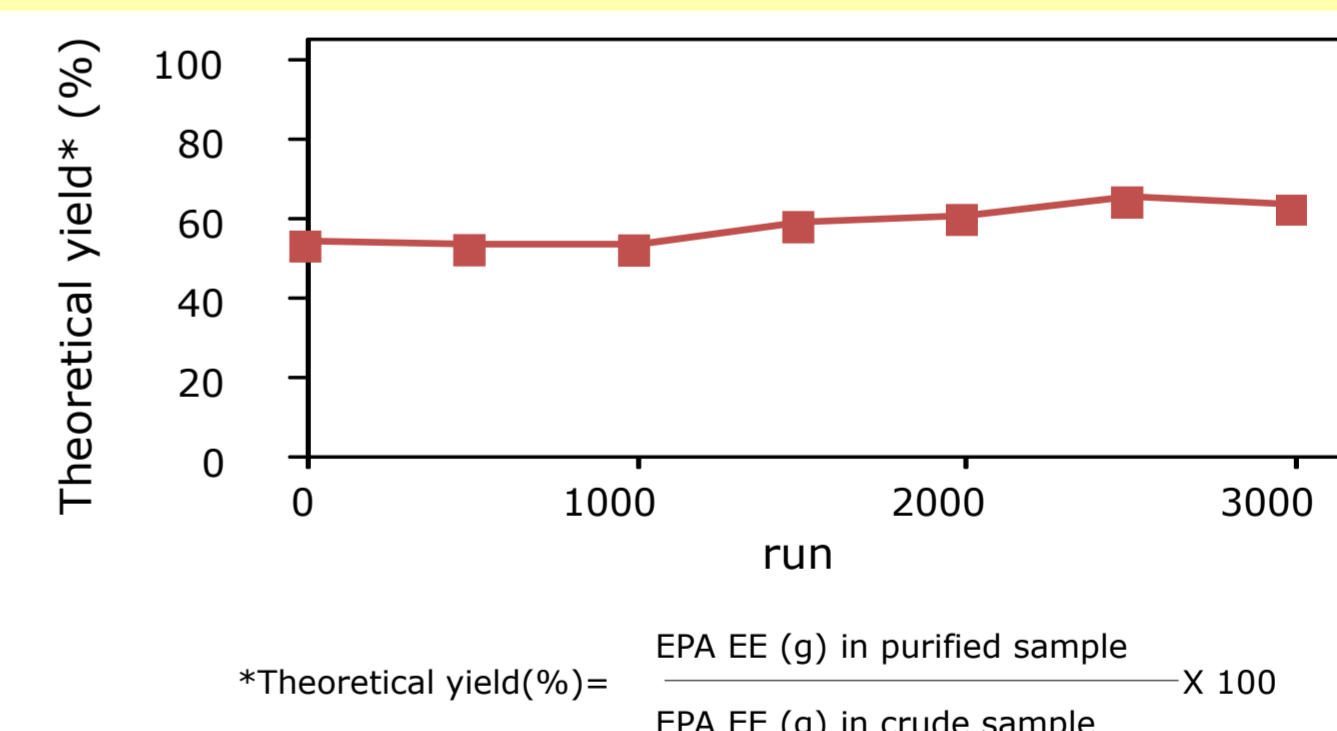
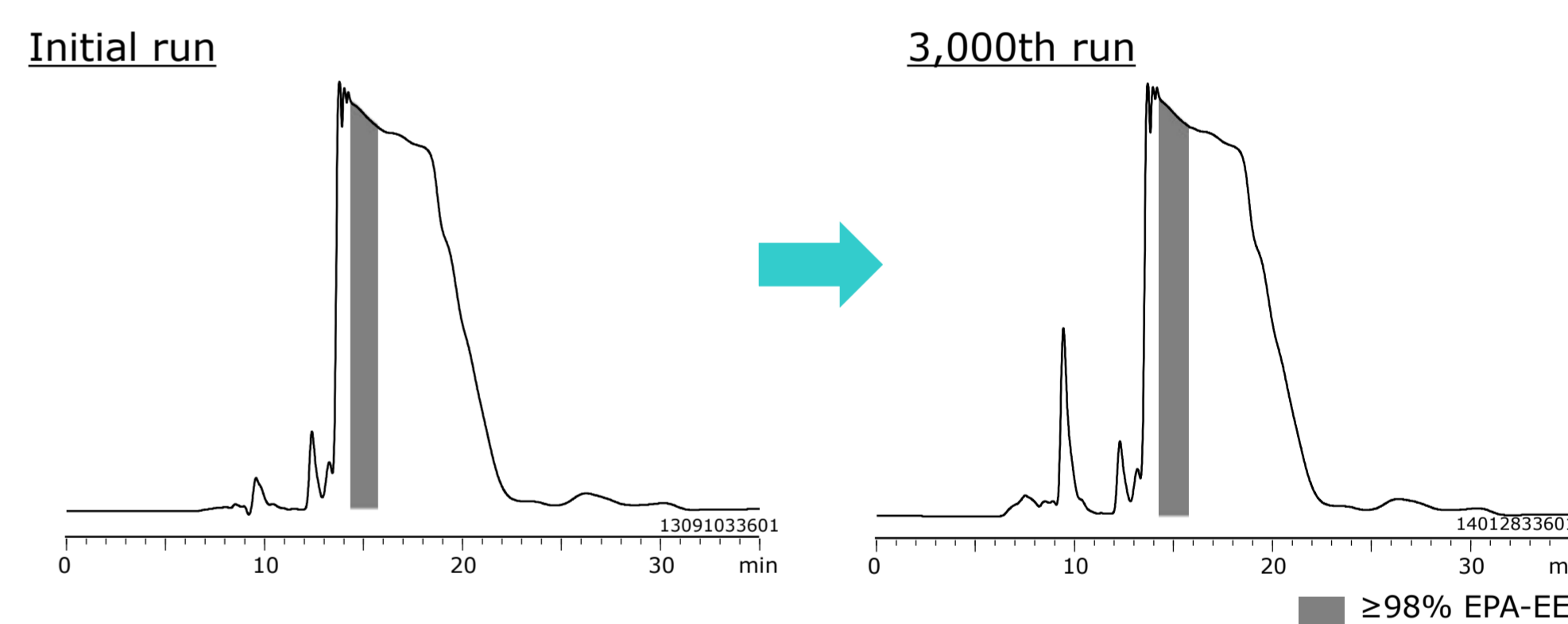
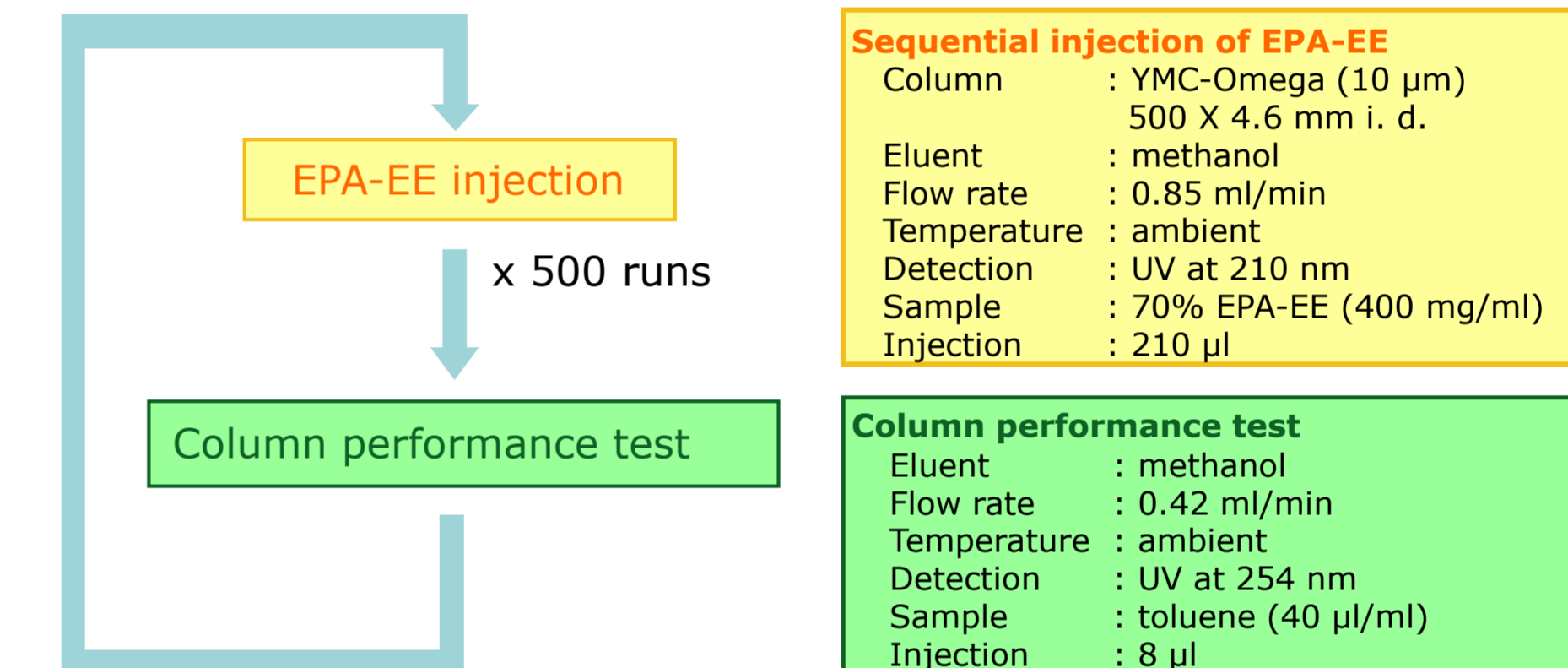


Although the yield per one run was greater than that at doubled flow rate, the productivity per unit time was 45% lower. The normal flow rate is not as good as the doubled flow rate in terms of productivity per unit time. However, high yield is effective for a case where yield has high priority, such as amount of raw material is limited or a cost of raw material is expensive.

In the case that flow rate was doubled, the theoretical yield was dropped by 7%. Nevertheless, the cycle time became half and productivity of EPA-EE per unit time increased by 1.8 times compared to normal flow rate. The production at doubled flow rate will result in remarkable reduction of operation days and improve productivity per year while only 5-10% more solvent and raw material is needed. The method developed here can be scaled up to a larger column, and therefore, it is ideal for a large-scale purification of high purity EPA-EE.

Stability study of YMC-Omega

Test procedure



Even after 3,000 sequential injections YMC-Omega provided outstanding stability and reproducibility on purification of EPA-EE. The theoretical yield has not been changed over 3,000 injections. Moreover, pressure increase was less than 10% during this sequential injections without any column cleaning with other solvent after each purification run. YMC-Omega, which has high durability, can achieve longer column lifetime and reduction of production cost.

Conclusions

- YMC's novel packing material, YMC-Omega, of which particle characteristics are specially designed for highly efficient purification of EPA ethyl ester (EPA-EE), exhibited excellent loadability and yield.
- Combination of YMC-Omega and optimized method for high purity and a large scale EPA-EE purification offers the most cost effective production.