

Introduction

Silica reversed phase chromatography (RPC) is a popular purification tool for peptide-based therapeutics such as insulins and GLP-1 compounds. In many cases, high pH conditions are desirable in these chromatography process such as column regeneration by 0.1M NaOH solution, where conventional silica media suffers short lifetime. Hybrid silica (such as XBridge) are well known for high pH stability in HPLC applications, but not available for process scale chromatography, presumably due to 1) low surface area of hybrid silica particles; 2) manufacturing capacity; 3) high cost of manufacturing such hybrid particles. Other companies developed hybrid coating on porous silica particles. Those hybrid-coated particles have improved lifetime over silica particles at high pH, but still are not as stable as hybrid particles. There is a big need for bulk media with high performance and much longer lifetime for purification of peptide-based drugs.

NanoMicro Technology is the only company that commercializes monodispersed silica bulk media for separation and purification process. Our innovative Precisely Controlled Silica (PCS) technology allows us to manufacture monodispersed silica particles in large quantity and low cost (several hundred kilograms a batch with almost 100% yield). Based on the PCS technology, we have recently developed monodispersed hybrid silica – UniHybrid Eterne to meet the demands of high performance RPC media for high pH tolerance.

Introduction of UniSil Revo and UniHybrid Eterne

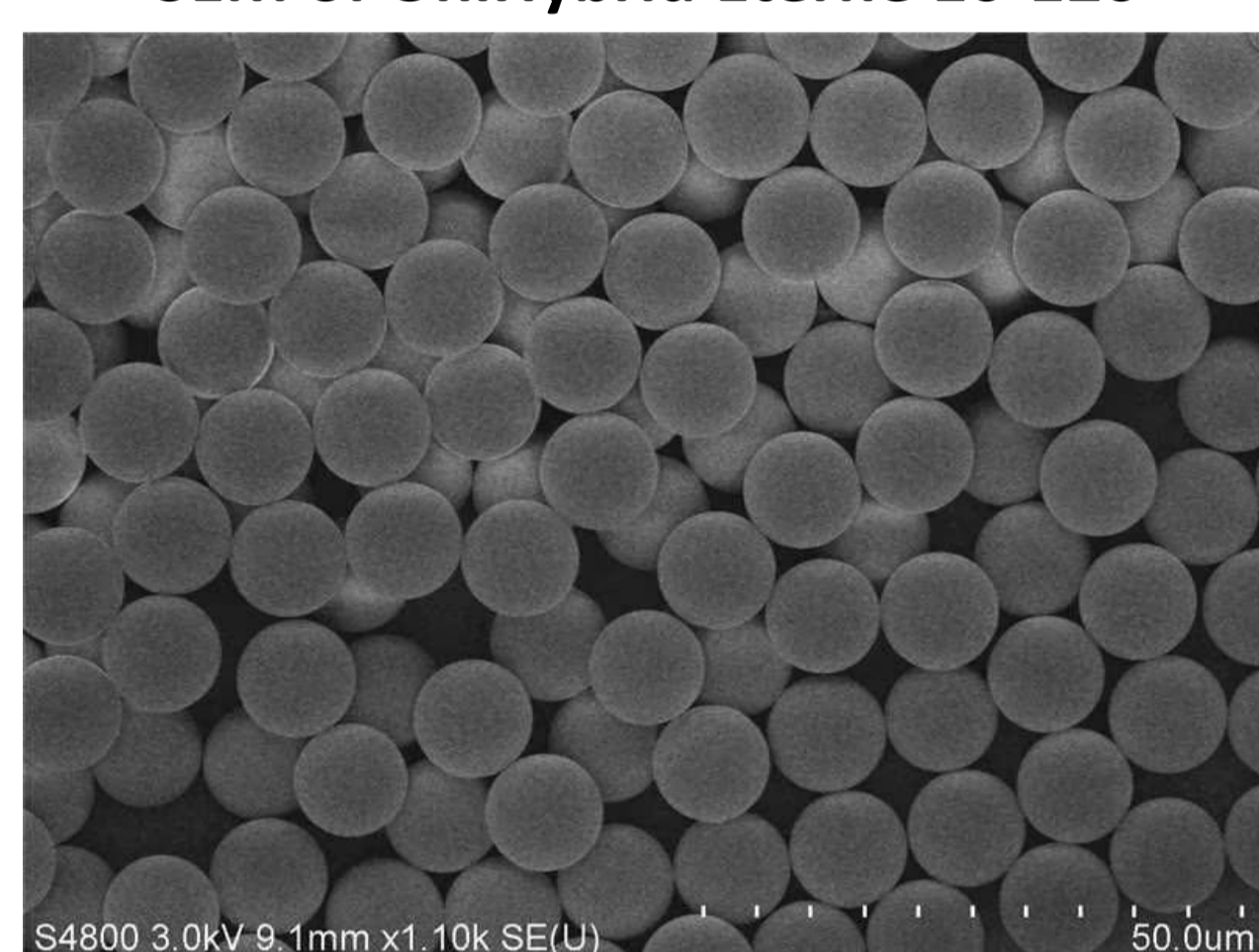


- Next generation of our UniSil particles with optimized pore structure and thicker pore walls for improved lifetime.
- Better selectivity and resolution of impurity peaks.
- High yield and high purity for insulin and GLP-1 purification.
- Bulk monodispersed hybrid silica particles for purification market.
- Same mechanic strength as silica particles, and **>10x better chemical lifetime at pH 13**.
- At high pH, UniHybrid Eterne provides different selectivity to separate impurities that are hard to separate at low and mid pH.

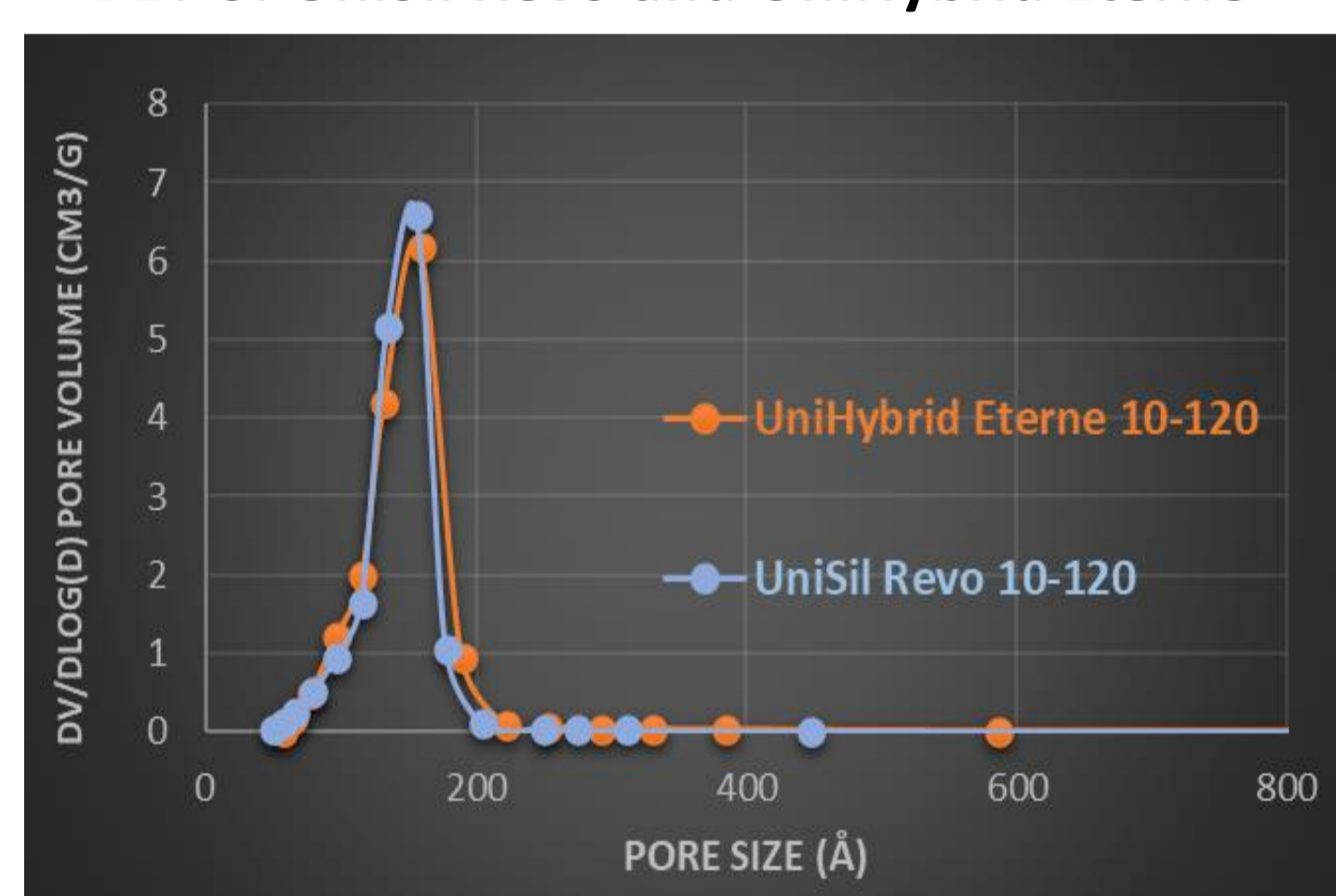
Physical Properties of UniHybrid Eterne 10-120

Particles	Particle size (µm)	Pore size (Å)	Ave. SA (m ² /g)	Pore volume (ml/g)	pH range	Max. pressure (bar)	Phases available
UniSil Revo	10	120	320	1.10	2-9	600	C18, C8, C4
UniHybrid Eterne	10	120	320	1.20	1-13	600	C18, C8

SEM of UniHybrid Eterne 10-120



BET of UniSil Revo and UniHybrid Eterne



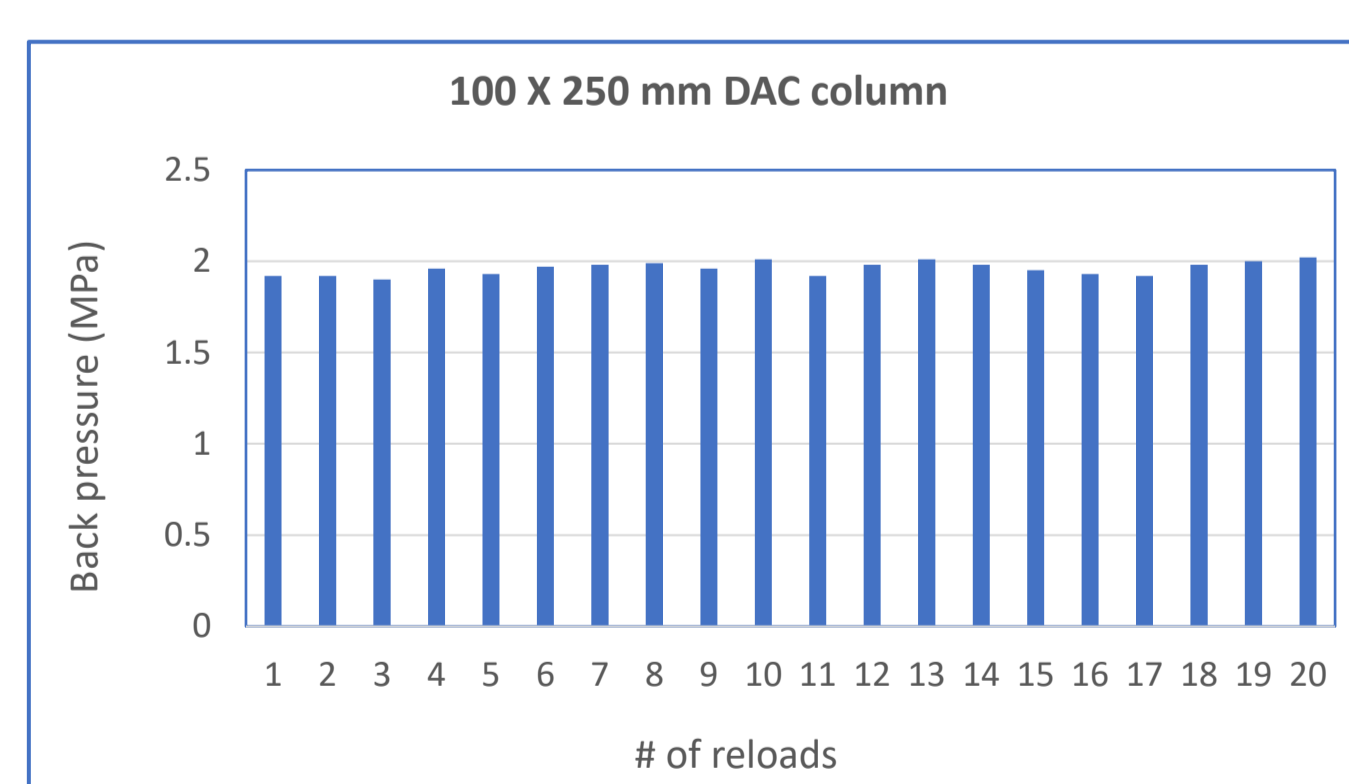
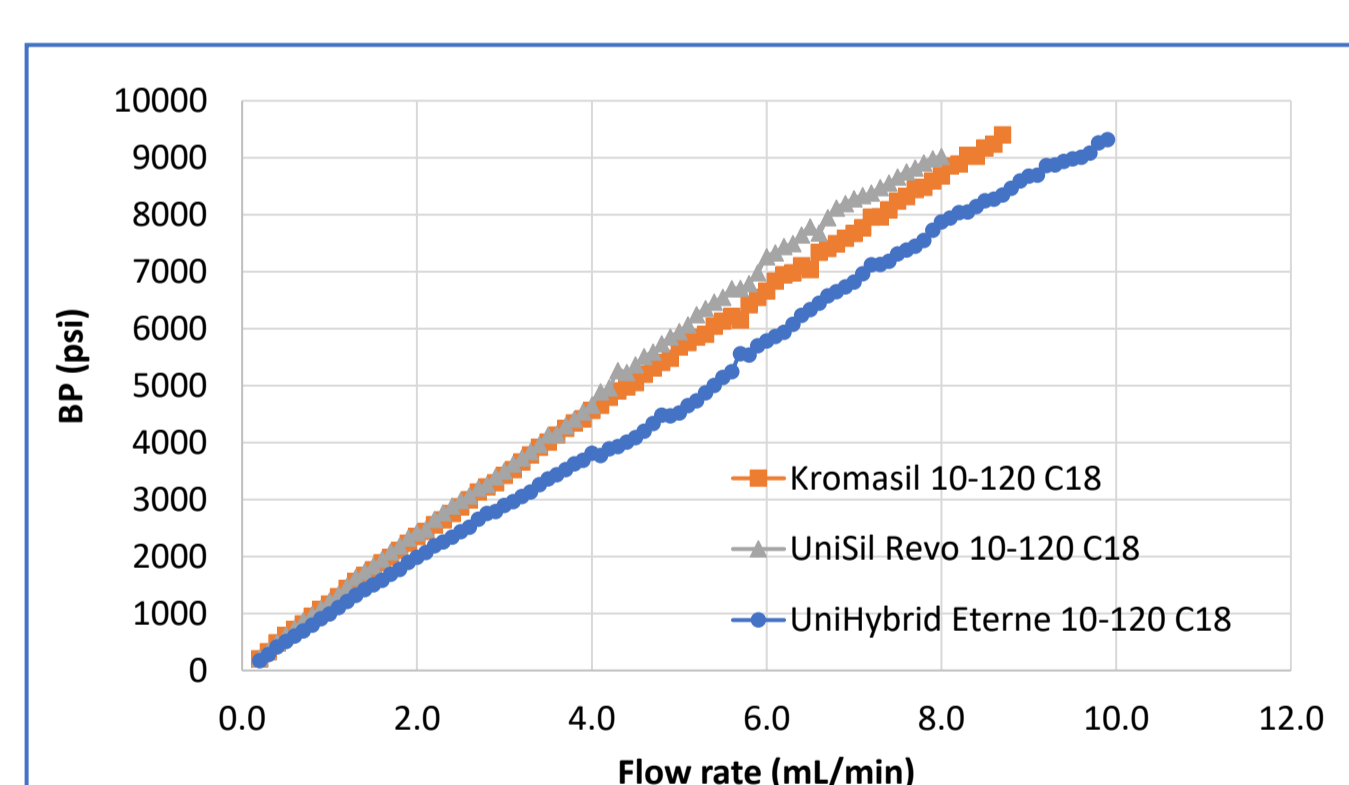
Mechanical Strength Test

Test method:
Column: 2.1 x 150 mm
Mobile phase: ethanol
Flow: from 0.2 ml/min to 10 ml/min

100 x 250 mm DAC Column

- Column loading:
 - UniHybrid Eterne 10-120
 - Weight: 750g
 - Loading pressure: 80 bar
- Test method:
 - Mobile phase: ethanol
 - Flow: 200 ml/min
 - Injection: 0.1% CV
 - Sample: 2% toluene
 - Detector: 254 nm

Both UniSil Revo and UniHybrid Eterne are able to stand up to 9000 psi (600 bar).



Chemical Stability at High pH of UniHybrid Eterne 10-120

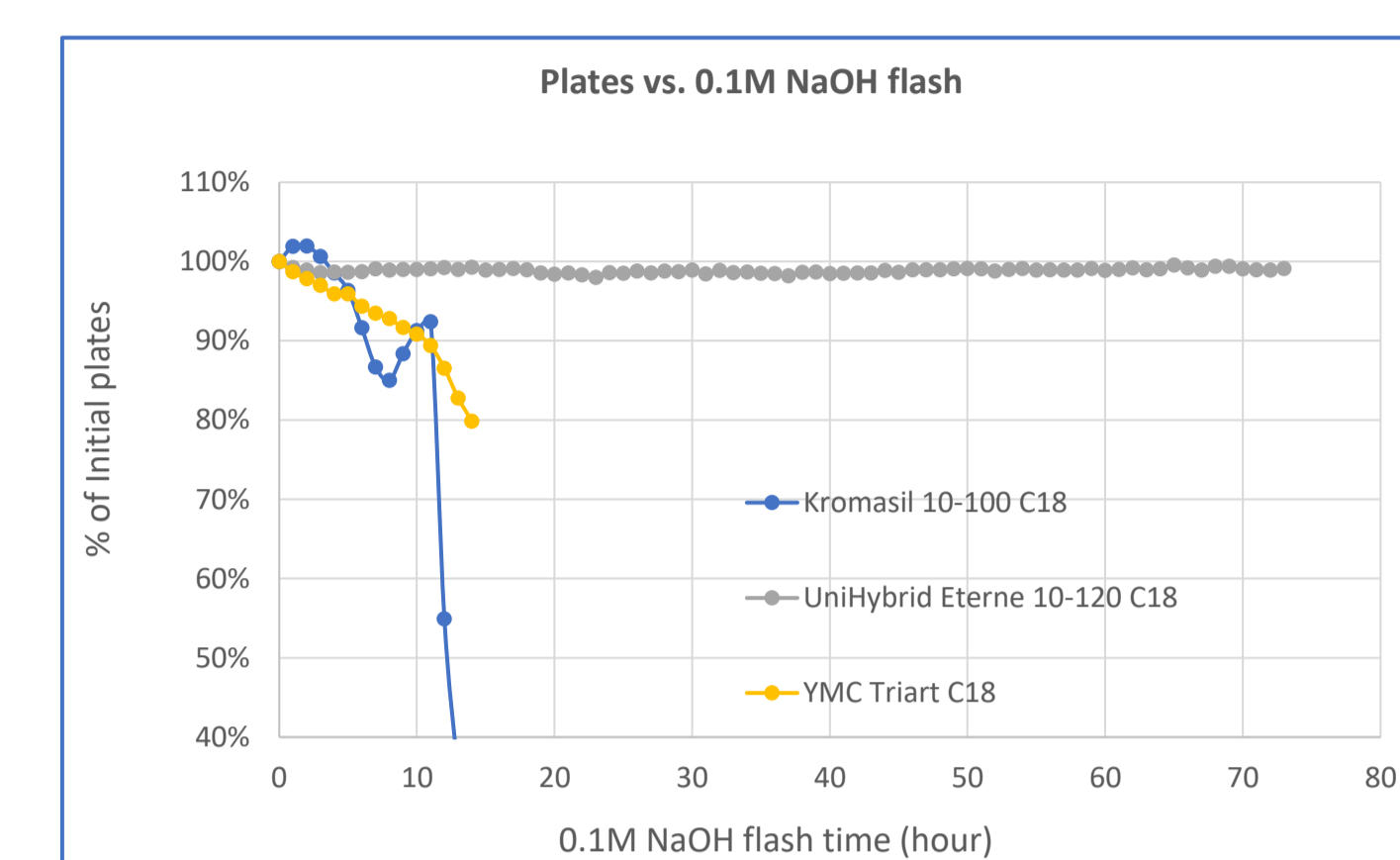
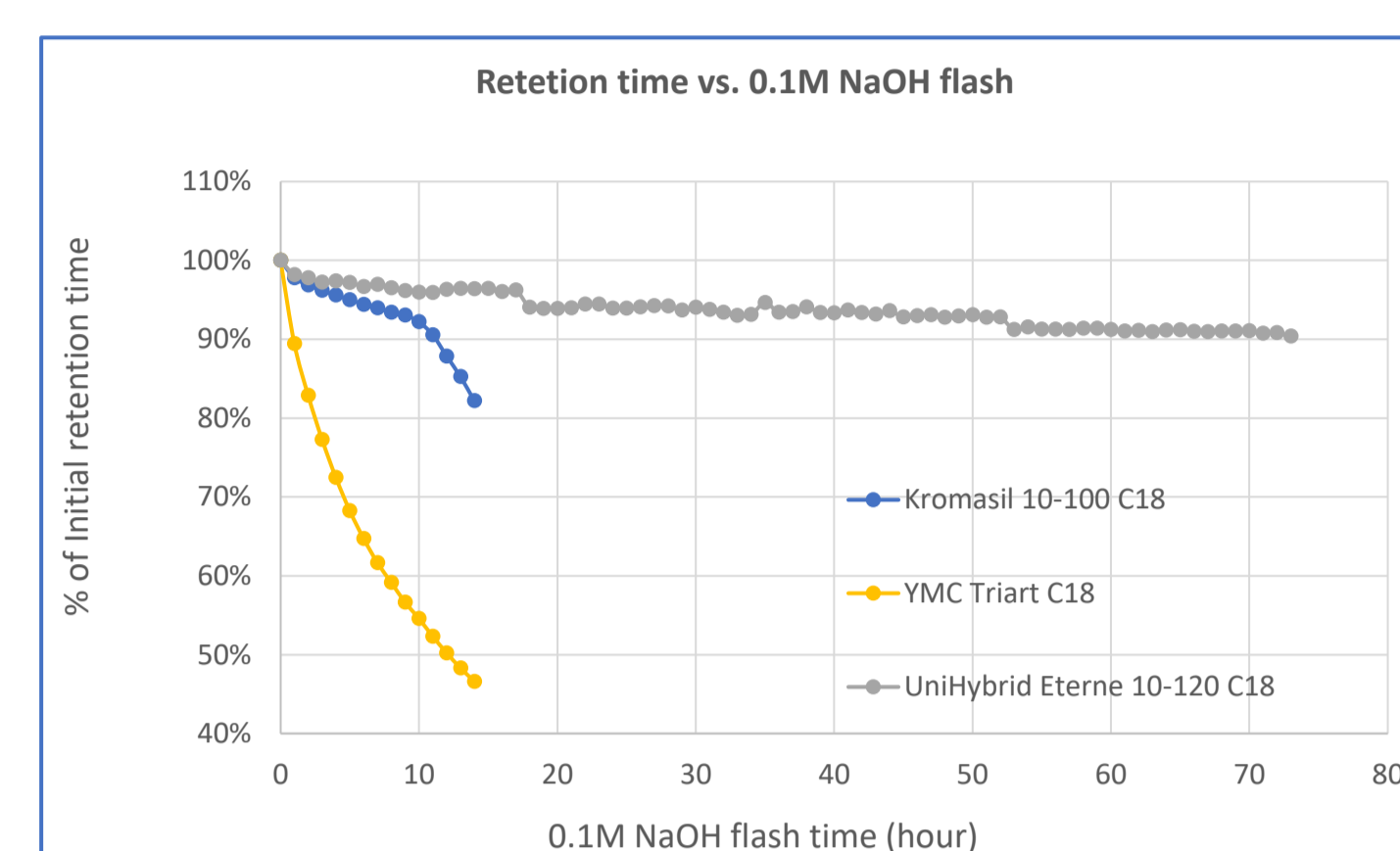
Column QC test method:

Column: 4.6 x 150 mm
Mobile phase: 60% ACN/40% water
Flow: 1.0 ml/min for 20 minutes
Detector: 254 nm
Temperature: 30°C
Sample: Urasil, Dimethyl phthalate, Naphthalene

High pH flash method:

Mobile: 50%/50% Ethanol/0.1M NaOH, pH=13.0
Flow: 1.0 ml/min for 60 minutes
Detector: 254 nm
Temperature: 30°C
No sample injection

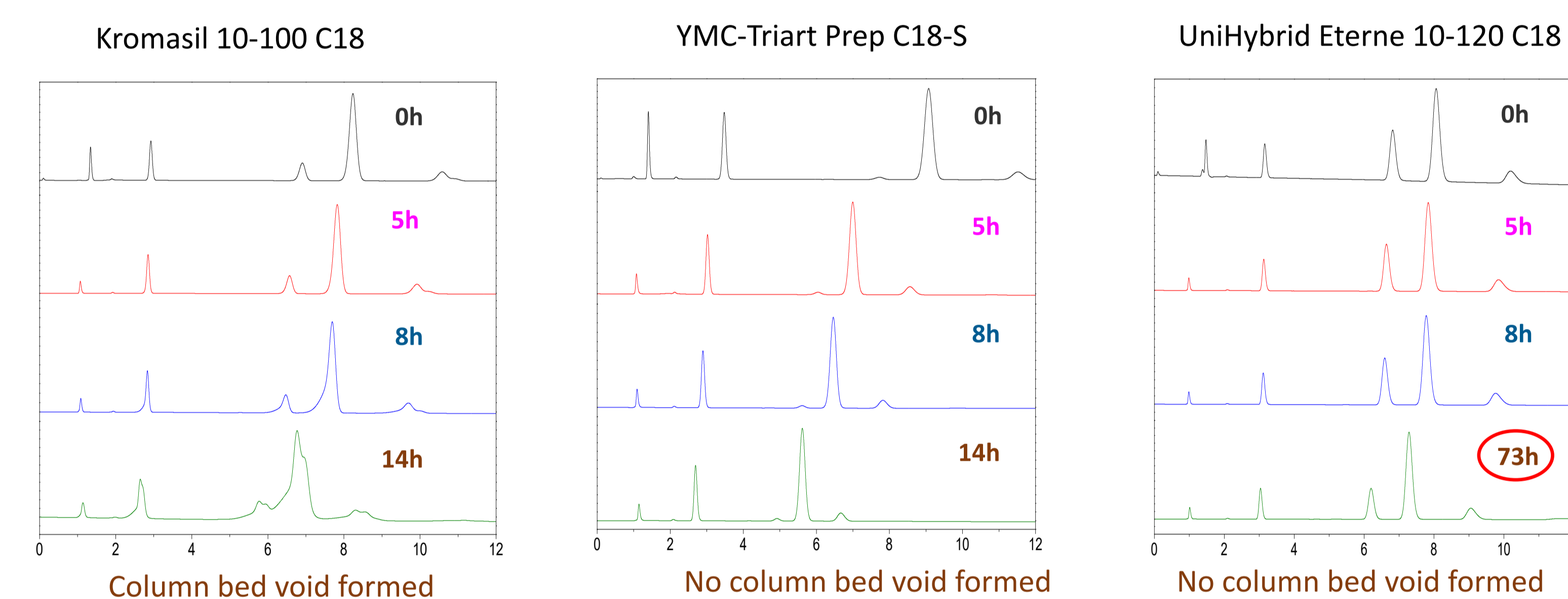
Testing cycle: Repeat column QC method and high pH flash method



UniHybrid Eterne 10-120 C18 retention time dropped only 8% after 73 hours flash in 0.1M NaOH

Comparison of C18 Lifetime at 0.1M NaOH Flash

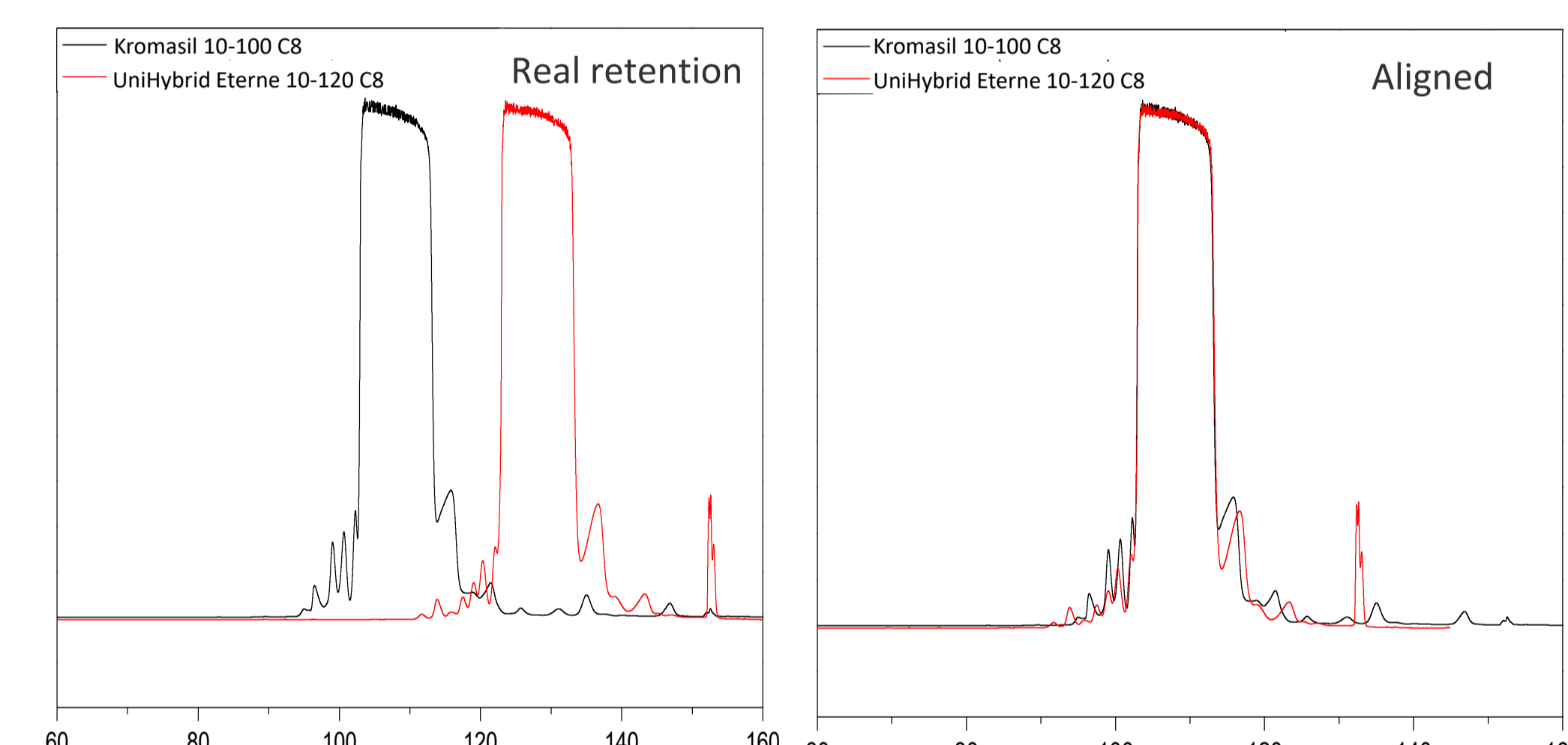
Comparing to hybrid coating particles such as YMC Triart C18 and Kromasil C18, UniHybrid Eterne 10-120 C18 showed much better lifetime in pH 13 condition regarding retention time and plates.



Insulin Purification

Method:
Column: 4.6 x 250 mm; C8
Mobile phase: A: buffer solution; B: ACN
Flow: 0.42 ml/min
Sample loading: 60 mg (14 mg/mL-CV)

Time	A	B
0	85	15
10	85	15
10.01	67	33
90	47	53
90.1	0	100
110	0	100

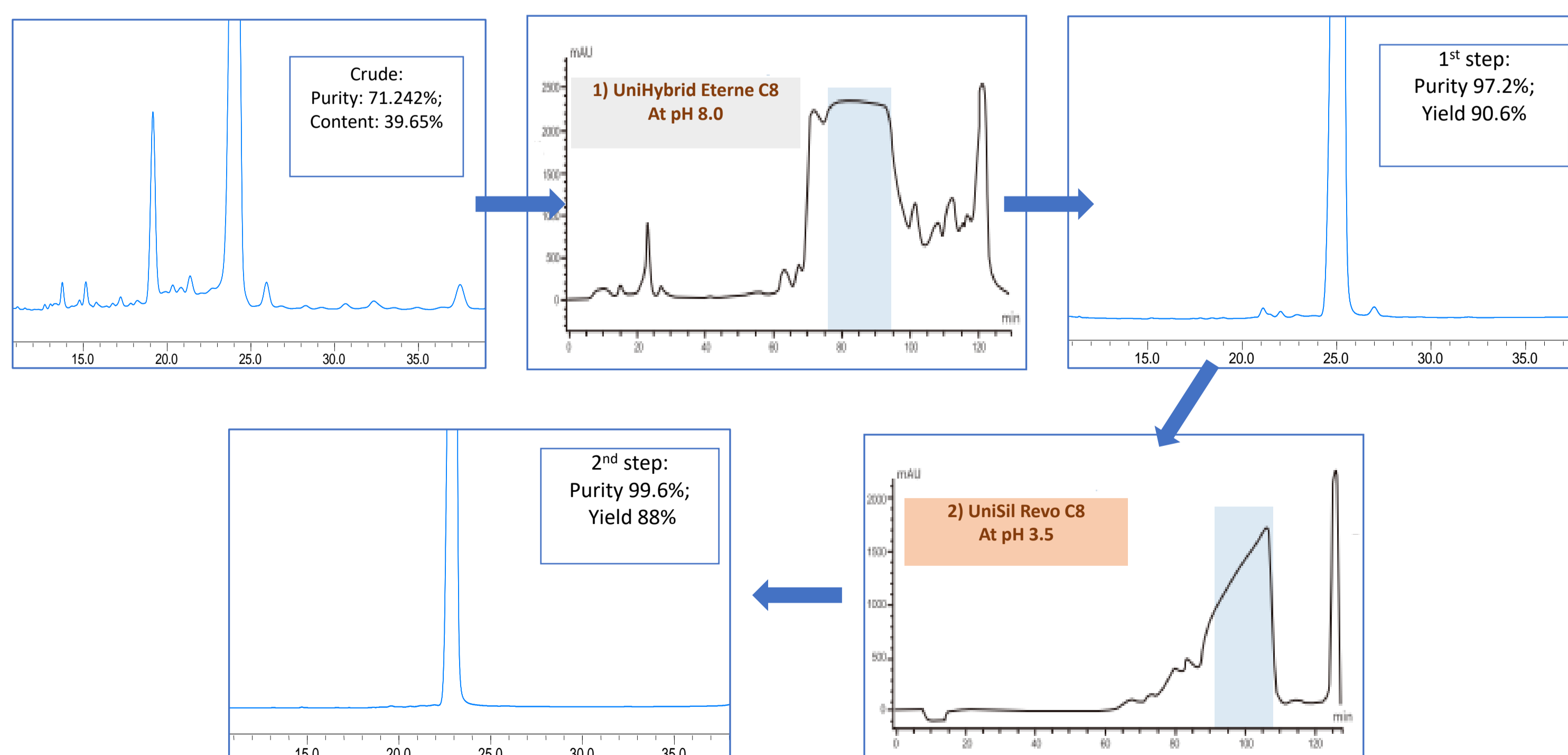


UniHybrid Eterne 10-120 C8 matches Kromasil 10-100 C8 performance

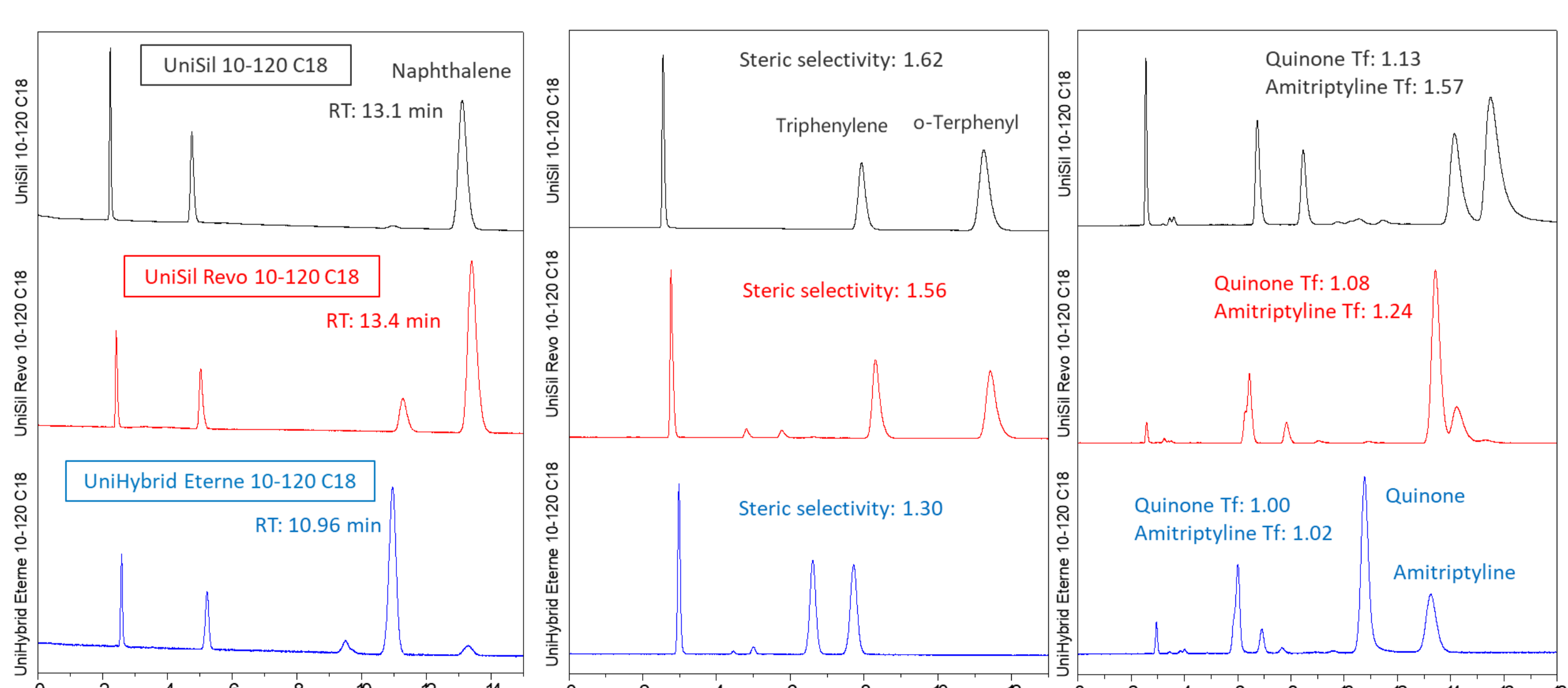
Semaglutide Purification

Strategy: A two-step purification process of semaglutide

- Purification of semaglutide under basic condition using UniHybrid Eterne C8;
 - 1st purification sample is usually dirty which requires more 0.1M NaOH washings/regenerations;
 - UniHybrid Eterne C8 provides higher yield under basic condition and excellent chemical lifetime for 0.1M NaOH.
- Purification under acidic pH 3.5 condition using UniSil Revo C8.
 - 2nd purification sample is clean which requires less 0.1M NaOH regenerations;
 - UniSil Revo C8 provides the better purity and yield.



Chromatographic Properties



Summary

- We have developed monodispersed UniHybrid Eterne for purification market, especially for insulin and GLP-1 purification.
- UniHybrid Eterne provides comparable purity and yield as silica based particles, but has 10x better chemical lifetime at pH 13 condition than silica based particles.
- By combination of UniHybrid Eterne and UniSil Revo, we provide a better solution for GLP-1 purification.
 - Using UniHybrid Eterne in the 1st step to ensure longer column lifetime and high yield;
 - Using UniSil Revo in the 2nd step to get high purity of the products.
 - Longer lifetime; better yield; higher purity.