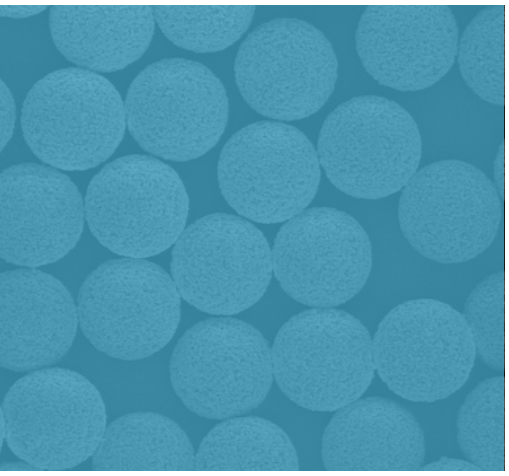
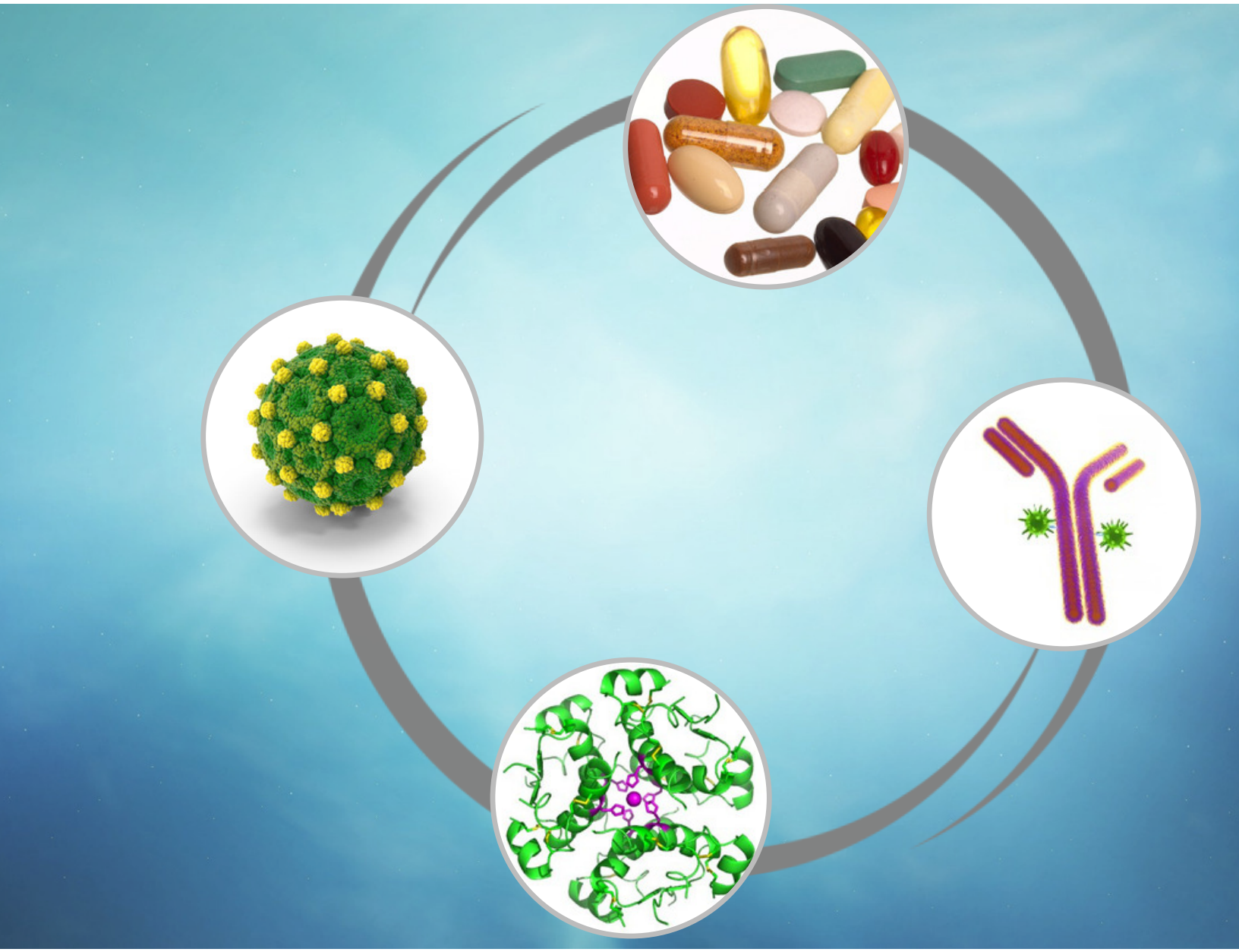
Sepax Technologies



Monomix™ IEX

ION EXCHANGE CHROMATOGRAPHY

POLYMERIC PROCESS MEDIA FOR
BIOMOLECULE SEPARATIONS



Our Specialty

Sepax Technologies, a Delaware US-based company, provides cutting edge products and services for liquid chromatography (LC). Sepax specializes in the development and manufacture of LC analytical, preparative and process separation & purification columns, bulk resins and systems in a wide range of modalities, such as SEC, IEX, HIC, Affinity, and RP.

Sepax also provides LC services, including analytical testing, method optimization, purification, custom resin development, and ligand immobilization. Certified to the ISO 9001-2015 standards, Sepax focuses on customer & market needs, and is continuing to expand its presence and supply chain around the globe in three business platforms: Analytical Chromatography, Industrial Purification and Medical Diagnostics.

Our Commitment

At Sepax, we create value through serving customers' needs and solving their chromatographic separation and purification challenges. Through innovative technologies and solution-based approaches, Sepax delivers products and services that build lasting relationships with customers, achieving a strong leadership role in the industry. At Sepax, we firmly believe that there is nothing too complicated or challenging for us to consider.

Our Strategy

Whether you are conducting analytical research, in need of customized resins, or scale-up purification, Sepax Services offers unmatched technical capabilities and expertise. Working in tandem with our technical team and our customers, Sepax offers highly individualized services to meet your specific requirements, achieving project goals in an efficient and cost-effective manner.

Monomix™ IEX

Introduction

Ion exchange (IEX) chromatography enables the separation of native biological samples without disruption of high order structures. It has been widely used in the analysis and separation of ionizable pharmaceutical molecules. The method can be applied to all stages of the biological sample purification process. Currently commercially available IEX media are mostly based soft agarose and polymethacrylate, which only accommodates low pressure operation.

Sepax has developed alternative polymethacrylate-based ion exchange media, which stands higher flow rate applications. Sepax developed narrowly dispersed polymethacrylate-based ion exchange process media. The particle size of the resin is 30 μm and 60 μm with an average pore size of 500 Å and 1000 Å. The highly uniformly dispersed resin has a narrow particle distribution of D90/D10<1.3, as shown in Figure 1. The base resin is made of hydrophilic polymethacrylate. On the resin surface is covalently linked with different ion exchange functional groups, strong cation exchange group (sulfonic acid), strong anion exchange group (trimethyl quaternary amine) and weak anion exchange group (diethylamine), as shown in Figure 2.

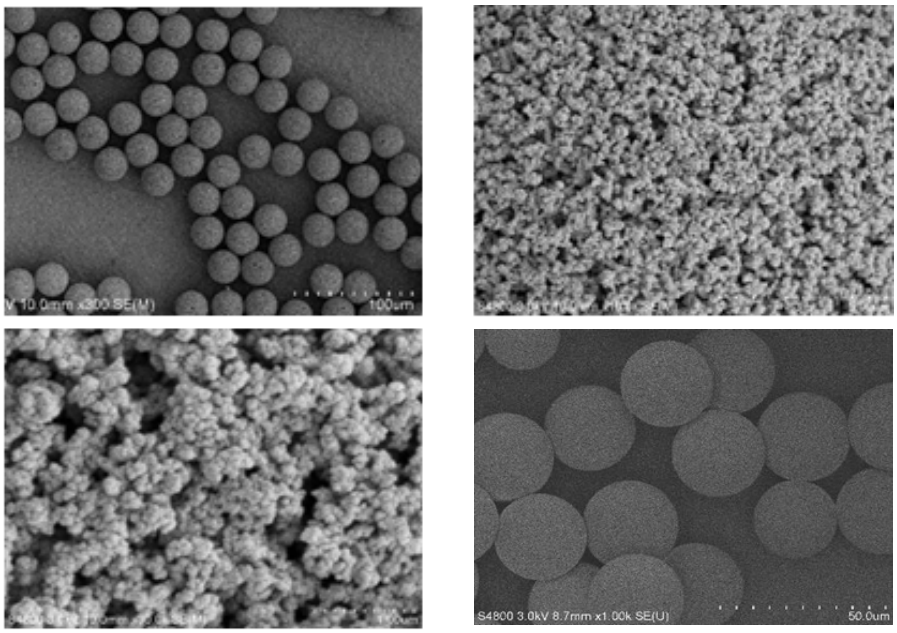


Figure 1. SEM images of Monomix MC30 Resin. The highly uniformly dispersed resin has a narrow particle distribution of D90/D10<1.3.

Features

- Monomix IEX resins are narrowly dispersed particles
- Well controlled pore structure
- Rigid beads can be operated at higher flow rates and higher pressure
- High dynamic binding capacity and high loading capacity
- High separation efficiency and resolution
- Wide pH range

Structure of Monomix MC30

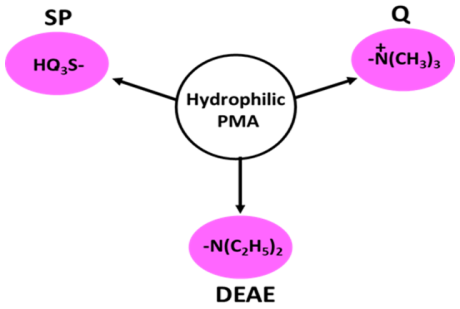


Figure 2. Monomix HC/MC30 IEX resin structures. Strong cation exchange group is sulfonic acid. Strong anion exchange group is trimethyl quaternary amine, while weak anion exchange group is diethylamine.

Resin Technical Specifications

Depending on the resin surface structure and ion exchange group density, the dynamic binding capacity (DBC) of the resins varies as shown in the table below. High DBC is achieved with 105 mg Lys/mL for strong cation exchange SP resin and 100 mg BSA/mL for strong anion exchange resin Q.

Resin Type	Monomix HC-Q			Monomix HC-DEAE			Monomix HC-SP		
Matrix	Hydrophilic polymethacrylate								
Functional Group	-N+(CH3)3			-N(C2H5)2			-SO3H		
Particle Size (μm)	15	30	60	15	30	60	15	30	60
Pore Size (Å)	1000			1000			1000		
DBC* (per mL resin)	≥ 95mg BSA	≥ 90mg BSA	≥ 70mg BSA	≥ 90mg BSA	≥ 90mg BSA	≥ 80mg BSA	≥ 110mg Lysozyme	≥ 90mg Lysozyme	≥ 90mg Lysozyme
Maximum Linear Flow Rate (cm/h)	1000								
Operation Temperature (℃)	≤ 40								
pH Range	2-12								
Maximum Pressure	≤ 1 MPa (10 bar)								
Compatible Mobile Phases	Compatible with aqueous solution, a mixture of water and acetonitrile, ethanol, etc. Typical buffers: Tris, phosphate, and acetate.								
Storage	50% (v/v) in 20% ethanol								
Regeneration	1-2 M NaCl								
CIP	0.5 M HCl or 0.5-1.0 M NaOH. Impurities with strong hydrophobic binding can be cleaned with 0.1-1% Tween or Triton X-100								

Flow- Pressure Characteristics

The resins have shown excellent flow-pressure characters as shown in **Figure 3**. HC resins showed higher back pressure than MC series, due to the resin surface structure difference.

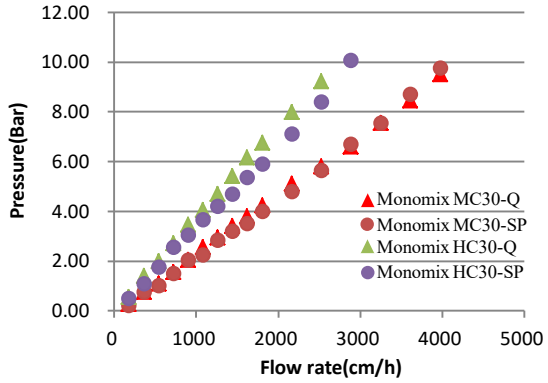
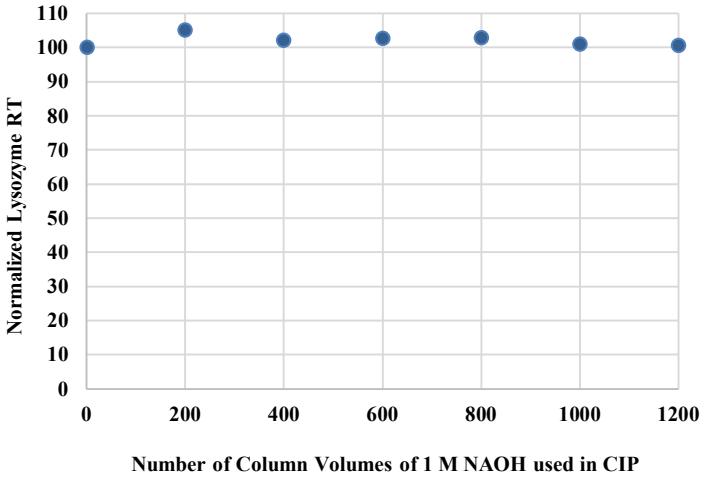


Figure 3. Back pressure vs. linear flow rate for Monomix IEX resins.

Instrument: Sepax FPLC Generik HP36
Column: 10 x 150 mm
Mobile Phase: 20 mM Sodium Phosphate (pH 7.0) for SP columns, 50 mM Tris (pH 8.0) for Q columns
Column Temperature: Ambient

CIP Effects on Lysozyme Retention Time

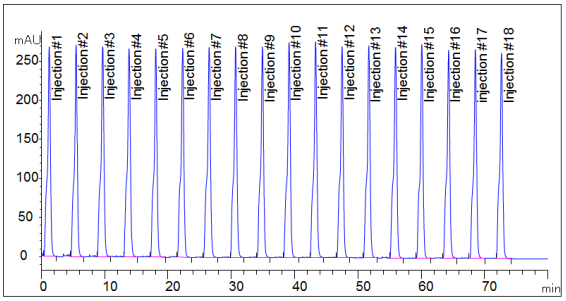


Resin: Monomix HC30-SP (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: A: 20 mM Sodium Phosphate (pH 6.0) B: A + 1.0 M NaCl
Flow Rate: 0.4 mL/min (144 cm/h)
Detector: UV 214 nm
Column Temperature: RT (20°C)
Injection Volume: 20 µL
Sample: Lysozyme, 1 mg/mL in water
Lysozyme Test: Gradient 0-75% B in 10 min
CIP: 1.0 M NaOH

Figure 4. The Monomix MC resin not only has higher physical strength, but shows caustic stability towards 1.0 M NaOH, which is routinely used in CIP. As illustrated in **Figure 4** there is negligible change in the retention time of lysozyme throughout CIP with 1.0 M NaOH for 1,200 column volumes.

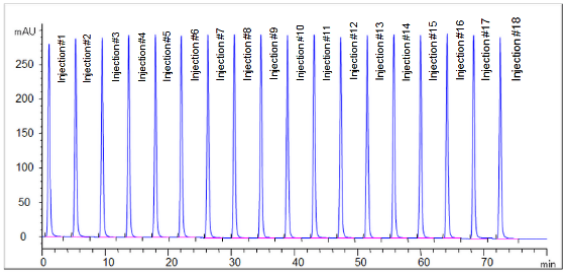
Non-Specific Binding Test from Monomix HC30-SP and Q Resin

The surface of Monomix IEX resin contains multi hydrophilic domains which minimizes the nonspecific binding of the biological analytes with the resins. As exemplified in **Figures 5 and 6**, both Monomix HC30-Q and SP showed minimal NSB toward Lysozyme and Bovine Serum Albumin (BSA).



Resin: Monomix HC30-SP (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: 20 mM Sodium Phosphate + 0.3 M NaCl, pH 7.0
Flow Rate: 0.5 mL/min (180 cm/h)
Detector: 214 nm
Column Temperature: RT
Injection Volume: 5 µL
Sample: BSA (1.0 mg/mL)

Figure 5. 18 injections of BSA through a Monomix HC30-SP column consecutively.

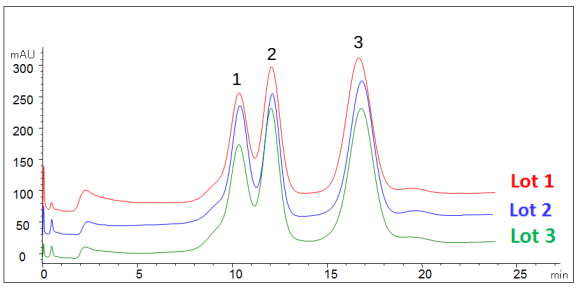


Resin: Monomix HC30-Q (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: 20 mM Sodium Phosphate + 0.3 M NaCl, pH 7.0
Flow Rate: 0.5 mL/min (180 cm/h)
Detector: 214 nm
Column Temperature: RT
Injection Volume: 5 µL
Sample: Lysozyme (0.5 mg/mL)

Figure 6. Consecutive 18 injections of Lysozyme through a Monomix HC30-Q column.

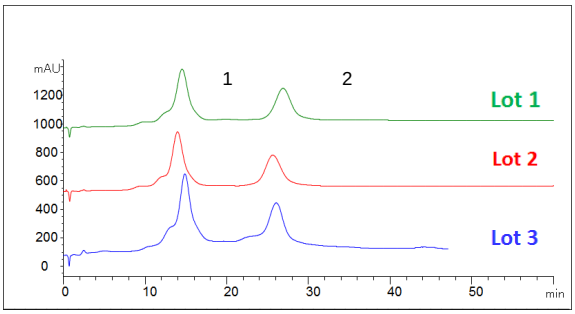
Lot-to-lot Consistency

Monomix IEX resins are manufactured with well controlled processes and are of high lot -to-lot consistency. Separations of standard protein mixtures on the Monomix IEX resins are illustrated in **Figure 7** & **Figure 8**.



Resin: Monomix HC30-SP (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: A: 20 mM Sodium Phosphate (pH 6.0) B: A + 1 M NaCl
Flow Rate: 1.0 mL/min (360 cm/h)
Gradient: 0-75% B 25 min
Detector: UV 214 nm
Column Temperature: RT
Injection Volume: 20 µL
Sample: 1) Ribonuclease A 2) Cytochrome C 3) Lysozyme (1mg/mL)

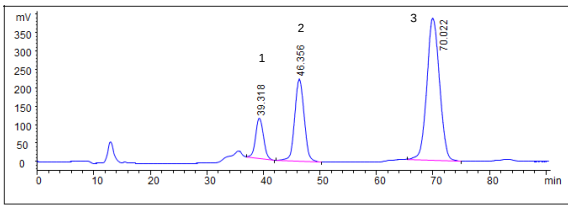
Figure 7. Lot-to-lot consistency for Monomix HC30-SP resins.



Resin: MonomixH C30-Q(30µ m, 1000Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase : A: 50 mm Tris pH 8.5 B: A + 0.5 M NaCl
Flow Rate: 1.0 mL/min (360 cm/h)
Gradient: 0-75% B 25 min
Detector: UV 214 nm
Column Temperature: RT
Injection Volume: 20 µL
Sample: 1) Ovalbumin 2) Trypsin inhibitor (5 mg/ml)

Figure 8. Lot-to-lot consistency for Monomix HC30-Q resins.

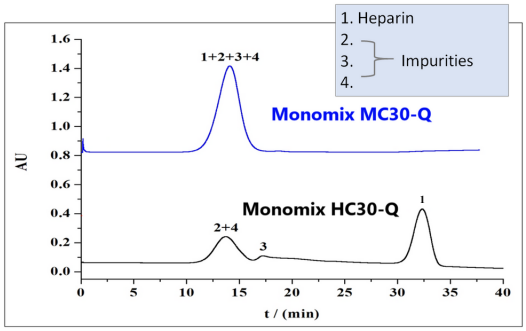
Easy to Scale Up for Monomix IEX Resins



Resin: Monomix HC30 SP (30 µm, 1000 A)
Column: 50 mm×220 mm FPLC
Instrument: Sepax FPLC Generik HP36
Mobile Phase: A: 20 mM PB (pH 6.0) B : 20 mM PB + 1 M NaCl (pH 6.0)
Flow Rate: 20 ml/min (61 cm/h)
Detector: 214 nm
Column Temperature: RT
Injection Volume: 4 mL
Sample: 1) Ribonuclease A (5 mg) 2) Cytochrome C (5 mg) 3) Lysozyme (10 mg)

Figure 9. Scale up from a small analytical column to a larger semi-preparative column is easy and straightforward. As shown in the **Figure 9**, a scaled-up separation of three protein mixture was achieved on a 50 x 220 mm column with high resolution and efficiency (chromatogram from an analytical column is not shown).

Separations of Heparin from Impurities in Production Mixture

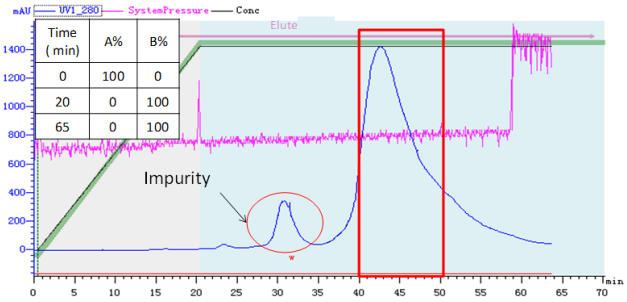


Resin: Monomix HC30-Q (30 µm, 1000 Å) and Monomix MC30-Q (30 µm, 1000 Å)
Column: 4.6 x 250 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: A: 4% NaH_2PO_4, pH 3.0 B: 4% NAH_2PO_4, 14% $NaClO_4$ pH 3.0
Flow Rate: 0.22 mL/min (80 cm/h)
Gradient: 25%-100% B from 10 min to 35 min
Detection: 202 nm
Injection Volume: 10 µL
Sample: Heparin (25 mg/mL) production sample

Figure 10. Sepax Monomix HC-30Q was successfully applied for the purification of heparin and related production impurities. This was further evaluated on analytical Ion Exchange (IEX) to confirm.

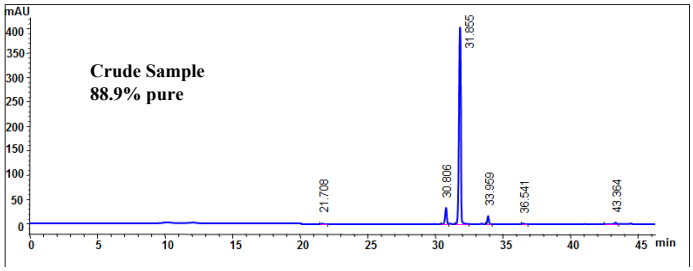
Purification of the ATP Analog on Monomix MC30-DEAE Column

Figure 11 shows the purification of an ATP analog sample by using Monomix MC30-DEAE. The purity of the crude ATP sample is 88.9% analyzed by HPLC, as shown in **Figure 12.** After one step of ion-exchange, it achieved purity of 98.6% with 98% recovery.



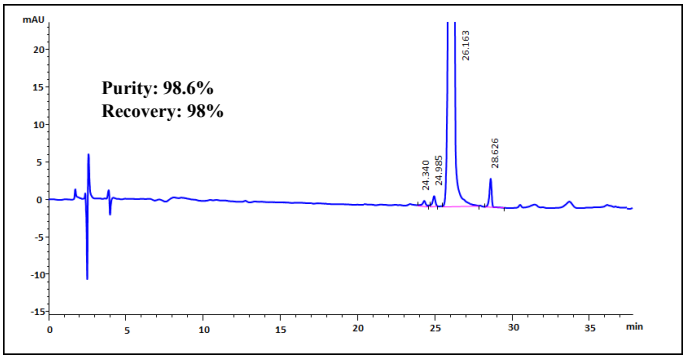
Resin: Monomix MC30-DEAE (30 µm, 1000 Å)
Column: FPLC 6.6x100 mm, AF
Mobile Phase: A: 20 mM Sodium Phosphate (pH 7.5) B: A + 1.0 M NaCl
Flow Rate: 1.0 mL/min (175 cm/h)
Detector: UV 280 nm
Column Temperature: Ambient
Injection Volume: 100 µL
Instrument: Sepax FPLC Generik HP36

Figure 11. Purification of an ATP analog sample by using Monomix MC30-DEAE.



Resin: GP-C18 (3µm, 120 Å)
Column: 4.6×250 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: A: Sodium Phosphate, pH 8.5 B: Acetonitrile
Flow Rate: 1.0 mL/min (360 cm/h)
Detector: UV 254 nm
Column Temperature: 30°C
Injection Volume: 2 µL
Sample: ATP Analog, 22 mg/mL in water

Figure 12. Purity analysis of crude ATP analog by HPLC.



Resin: GP-C18 (3µm, 120 Å)
Column: 4.6×250 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: A: Sodium Phosphate, pH 8.5 B: Acetonitrile
Flow Rate: 1.0 mL/min (360 cm/h)
Detector: UV 254 nm
Column Temperature: 30°C
Injection Volume: 2 µL
Sample: Purified ATP Analog, 22 mg/mL in water

Figure 13. Analysis of ATP purified sample after one step of ion-exchange with Monomix MC30-DEAE.

Ordering Information

Resin	Particle Size	Pore Size	Part Number
Monomix MC30-Q	30 µm	500 Å	280430500
Monomix HC30-Q	30 µm	1000 Å	280830950
Monomix MC30-DEAE	30 µm	500 Å	280530500
Monomix HC30-DEAE	30 µm	1000 Å	280930950
Monomix MC30-SP	30 µm	500 Å	280230500
Monomix HC30-SP	30 µm	1000 Å	280630950

Resin	Particle Size	Pore Size	Part Number
Monomix MC60-Q	60 µm	500 Å	280460500
Monomix HC60-Q	60 µm	1000 Å	280860950
Monomix MC60-DEAE	60 µm	500 Å	280560500
Monomix HC60-DEAE	60 µm	1000 Å	280960950
Monomix MC60-SP	60 µm	500 Å	280260500
Monomix HC60-SP	60 µm	1000 Å	280660950

Standard packing size: 1L, 5L, 10L, 25L, 50L, 100L

Additional pack sizes are available
Additional particle and pore sizes are available. Pre-packed stainless-steel columns for sample preparation and separation process development/ scale-up are available. Please contact your regional sales agent for more information.

Better Surface Chemistry For Better Separation

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