Application Handbook

Chromatography Solutions for API Manufacturers

www.phenomenex.com

phenomenex

Quality filtration products from Phenomenex offer a convenient way to clean up your samples - for improved analytical results with a simple method. Phenex syringe filters are designed for efficient and rapid filtration of almost any solution prior to analysis, and are optimized for superior flow rates and high throughput. Phenex offers a wide variety of membranes ideal for any application.

- High quality filtration product
- Increased column lifetime
- Less system downtime

The housing attaches to any standard Luer lock syringe, so the sample can easily be pushed through the membrane with minimal pressure. The result is a particulate-free eluent that is ready for use with HPLC, GC, or other analytical techniques.

Particulates Trapped

Particulate-free Sample

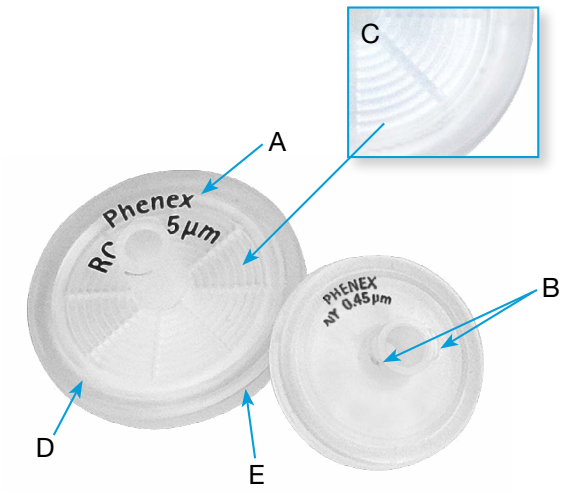
A Sample Preparation Solution

Filtration is one of the most popular sample preparation methods due to the ability to remove particulates before injection onto the column, the ability to use it in parallel with another sample preparation technique, and requires no method development.

Avoid Unnecessary Problems

Using low-quality, low-cost filters can lead to sample contamination by introducing filter membrane and housing extractables directly into your sample. This will negatively affect chromatography and can cause ghost peaks for future runs.

All Phenex filters are made with the highest grade materials available to remove any unwanted interferents.



Phenex Helps Reduce

Extraneous peaks	Sample co-elution
False quantitation	Instrument damage

Ensure Quality

A **Identification**
Membrane type and pore size are clearly marked on individual syringe filters

B **Luer Lock Inlet Tip**
Secures connections to prevent “blow off”

C **Sample Distribution Rings**
Creates even sample distribution for high sample flow rates

D **Medical Grade Polymer Housing**
Offers the most inert syringe filter and helps eliminate unwanted secondary interactions with the filter housing

E **Ultrasonically Welded**
Ensures robust housing - filter integrity

To learn more about sample preparation options, go to

www.phenomenex.com/samplepreparation

Which Filter Membrane Is Right for Me?

Phenex syringe filters are offered in a variety of chemically compatible membranes that are ideal for any application. Proper membrane and size selection are the keys to choosing the best product to maintain the integrity of your sample components as well as to protect your system from particulate contamination.

Select your filter in three EASY steps:

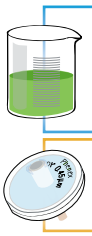
1. What is your sample volume?

≤ 2 mL Sample Volume	2 to 10 mL Sample Volume	10 to 100 mL Sample Volume
4mm Diameter	15mm Diameter	25 - 28m m Diameter

2. What is your LC column particle size?

> 3 µm	< 3 µm	OR
0.45 µm	0.20 µm	Viscous samples such as serum, plasma, or other biological matrices. Solutions with high particulate load (e.g., some environmental or food and beverage applications).
		Glass Fiber Filter with 0.45 µm membrane

3. What type of sample are you working with?



Aqueous			Solvents	
Solvents Mixtures	Tissue Culture Media, Buffers	Protein Analysis/ Biological Samples	Non-Aqueous	Aqueous Mixtures
↓	↓	↓	↓	↓
			Hydrophobic/ Strong Acids	Hydrophilic
			↓	↓
RC (Regenerated Cellulose)	**CA** (Cellulose Acetate)	**PES** (Polyethersulfone)	**PES** (Polyethersulfone)	**RC** (Regenerated Cellulose)

Need help? Try our Syringe Filter FInder.

Most Popular Filter Membrane Options

RC (Regenerated Cellulose)	PTFE, Teflon® (Polytetrafluoroethylene)
For Aqueous and Mixed Organic Solutions	**For 100% Organic Solutions**
A broad range of aqueous and mixed-organic solutions	Well-suited for the clarification of non-aqueous samples
Fast-flow and ultra-low protein and non-specific binding characteristics	Hydrophobic membrane, excellent for filtration of organic-based, highly acidic or basic samples and solvents
Broadly recommended as an excellent general purpose/high-performance sample filter for most applications	A hydrophobic membrane, that can be made hydrophilic by wetting with alcohol and then flushing with deionized water

Additional Syringe Filter Membranes

Membrane Types	Recommended Uses
PES (Polyethersulfone)	Polyethersulfone membranes exhibit very fast-flow and ultra-low protein binding characteristics. Phenex-PES membranes are typically broadly recommended for filtering critical biological samples, tissue culture media, additives and buffers.
NY (Nylon)	Nylon has inherent hydrophilic characteristics and works well for filtration of many aqueous and mixed-organic samples. In combination with a glass pre-filter (Phenex-GF/NY), this membrane is excellent for the filtration of particle-laden samples, such as foods and beverages, environmental, biofuels, and dissolution samples.
CA (Cellulose Acetate)	Cellulose Acetate (CA) membranes exhibit ultra-low protein binding and are broadly used in the filtration of biological samples. In combination with a glass pre-filter (Phenex-GF/CA), this membrane is excellent for filtration of tissue culture media, general biological sample filtration and clarification.
GF (Glass Fiber)	Glass Fiber (GF) filters are made of inert borosilicate glass and have a nominal 1.2 µm pore size. They are commonly used with highly viscous samples or samples containing high concentrations of particulate matter (e.g., food analysis, biological samples, soil samples, fermentation broth samples, removal of yeasts, molds, etc.).
PVDF (Polyvinylidene Fluoride)	Hydrophilic PVDF membrane provides high flow rates and throughput, low extractables, and broad chemical compatibility. This membrane binds less protein than nylon or PTFE membranes.

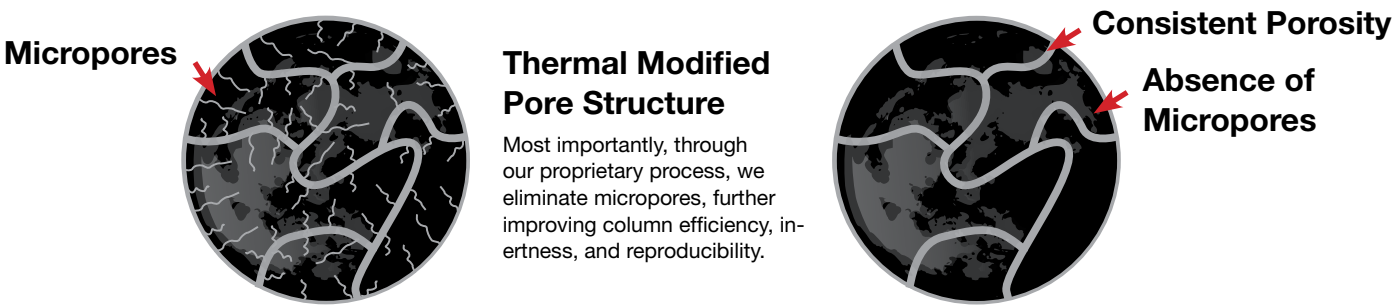
Need help? Try our Syringe Filter FInder.

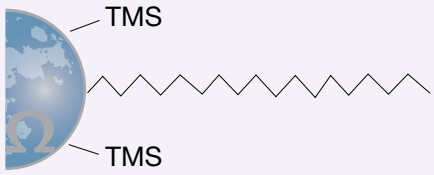
HPLC Solutions:
Thermally Modified Fully Porous particles

Luna Omega is a fully porous silica re-engineered for the 21st century. The thermally modified particles exhibit higher efficiency and greater inertness, providing highly efficient, high resolution separations with excellent peak shape.

The manufacturing process was specifically designed to control batch to batch reproducibility, providing a stable platform for method development and implementation of methods in QC environments across the lifespan of pharmaceutical products.

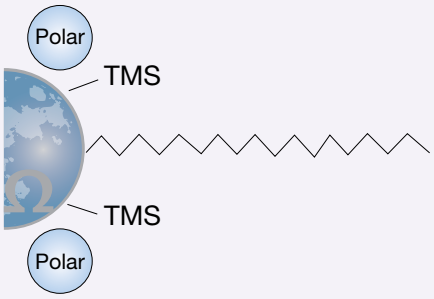
Thermal Modified Pore Structure





Luna Omega C18
Excellent first choice C18 column demonstrating high levels of inertness

- Gemifloxacin Mesylate and organic impurities
- Levothyroxine sodium and related substances



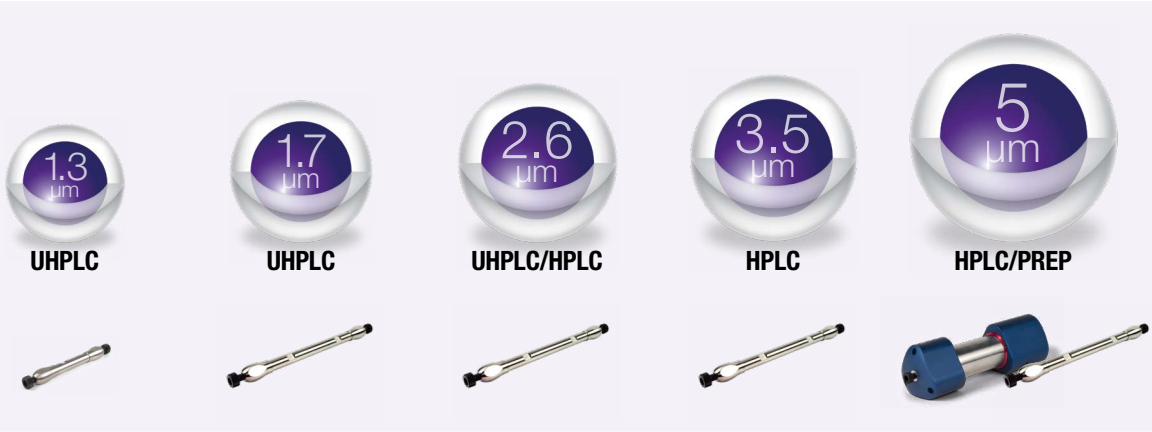
Luna Omega Polar C18
Novel C18 phase providing increased retention for both polar and non-polar analytes

- Paliperidone and organic impurities

Learn more about Luna Omega and how to order.

Kinetex™ Core-Shell Technology

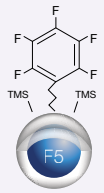
Phenomenex offers a wide range of columns, both high efficiency core-shell and fully porous materials. Our Kinetex range of core-shell selectivities are available in a range of particle sizes to match with the instrumentation you have, and have been proven to separate drug substances, related compounds and impuritiest as the resources below demonstrate.



The high efficiency delivered by Kinetex core-shell particles allow for high resolution separations, enabling the user to separate complex mixtures of API and impurities.

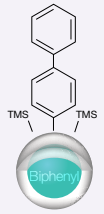
Kinetex F5 – a pentafluorophenyl stationary phase bonded to our high efficiency core-shell material. The phase provides excellent selectivity for halogenated, conjugated, isomeric or highly polar compounds

- Ezetimibe and organic impurities



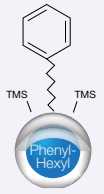
Kinetex Biphenyl – a 100% aqueous stable biphenyl phase bonded to core-shell particles. This stationary phase provides reversed phase selectivity for hydrophobic, aromatic and polar compounds

- Diazoxide and organic impurities



Kinetex Phenyl-Hexyl – this is a more hydrophobic phenyl phase due to the presence of the C6 linker. It provides aromatic interactions and hydrophobic interactions, providing an alternative selectivity to C8 or C18 phases.

- Miconazole Nitrate and organic impurities



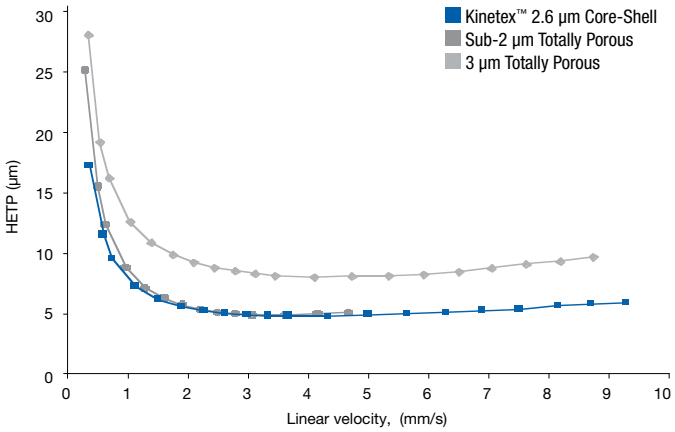
Learn more about Kinetex and how to order

Kinetex™ Core-Shell Technology

Core-shell vs. Fully Porous

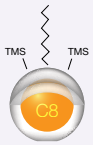
High Efficiency, High Density Particle

This van Deemter plot indicates that Kinetex core-shell particles exhibit higher plate count (lower HETP) than fully porous materials, allowing for the separation of complex mixtures.



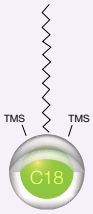
Kinetex C8 – a C8 (octyl) stationary phase, ideally suited for very hydrophobic compounds which might otherwise elute late on a C18 column.

- Furosemide and related substances



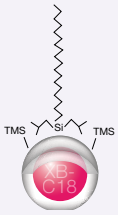
Kinetex C18 – All purpose C18 with excellent methylene selectivity

- Lamivudine and related substances
- Acetylsalicyclic acid, impurities and related substances



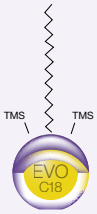
Kinetex XB-C18 – core-shell particles bonded with C18 chains with additional protective butyl side-chains

- Related substances of Clarithromycin on a Kinetex 3.5 µm XB-C18 column
- Albuterol organic impurities
- Atorvastatin related substances



Kinetex EVO – core-shell technology combined with organo-silica grafting, bonded with C18 to provide a robust stationary phase resistant to extremes of pH

- Ibuprofen related substances on fully porous and core-shell organo-silica HPLC columns



Learn more about Kinetex and how to order

The USP chapter 467 outlines the considerations and testing required by manufacturers of API and drug products. Testing is conducted using static headspace extraction followed by testing using a G43 or G16 stationary phase GC column.

- G43 – 6% cyanopropylphenyl-94% dimethylpolysiloxaine – ZB-624*PLUS*
- G16 – Polyethylene glycol compound (average molecular weight about 15,000). A high molecular weight compound of polyethylene glycol and a diepoxide linker – ZB-Wax*PLUS*

Technical note – TN-2074 Residual Solvents per USP 467 using Zebron™ ZB-624WAX*PLUS*™ and ZB-WAX*PLUS*™ GC Columns" provides details on:

- The classes of solvents
- Procedures A, B and C for the identification, confirmation and quantification of solvents

We have developed high performing columns with industry leading levels of inertness for this application:

- ZB-624*PLUS*™
- ZB-WAX*PLUS*™

Learn More about our GC Options.

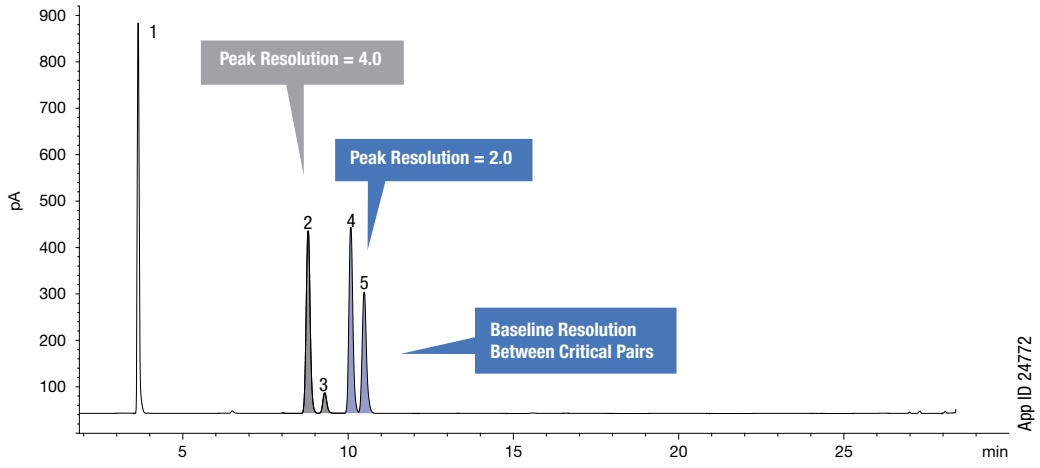
These Plus GC solutions provide improved performance thanks to increases in:

- Stability
- Inertness
- Reproducibility
- Low Bleed

Classification of Residual Solvents by Risk Assessment

Residual Solvent Class	Risk Assessment	Appropriate Analytical Procedure
Class 1	• Known/strongly suspected human carcinogens • Environmentaly hazardous • Solvents to be avoided	If Class 1 solvents are used or produced and are not removed by a process, then these solvents should be identified (Procedure A & B) and quantified (Procedure C)
Class 2	• Nongenotoxic animal carcinogens • Possible irreversible toxicity • Suspected reversible toxicity • Solvents to be limited	If Class 2 solvents are present at greater than the Concentration Limit specified by <467>, Option 1 or 2 limits, then these solvents should be identified (Procedure A & B) and quantified (Procedure C)
Class 3	• Solvents with low toxic potential • No health-based exposure limit [Class 3 residual solvents have PDEs of 50 mg or more per day]	If Class 3 solvent limit in an individual monograph is greater than 50 mg per day, then that solvents should be identified (Procedure A & B) and quantified (Procedure C)

USP Class 1 Standard Solution on Zebron™ ZB-624PLUS™ GC Column



Same conditions for all separations:

Column: Zebron ZB-624PLUS
Dimensions: 30 meter x 0.32 mm x 1.80 µm
Part No.: 7HM-G040-31
Injection: Split 5:1 @ 140 °C, 1 µL
Recommended Liner: Zebron PLUS Straight Z-Liner™
Liner Part No.: AG2-0A03-05 (for Agilent® & Thermo Scientific® systems)

Carrier Gas: Helium @ 2.2 mL/min (constant flow)
Oven Program: 40 °C for 20 min, to 240 °C at 10 °C/min
Detector: FID @ 250 °C
Sample 1. 1,1-Dichloroethene
2. 1,1,1- Trichloroethane
3. Carbon tetrachloride
4. Benzene
5. 1,2-Dichloroethane

Learn More about our GC Options.

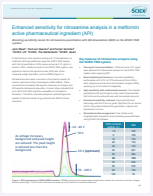
Nitrosamines are known to be carcinogenic and have the potential to be intermediates in organic synthesis. Due to their potent genotoxicity, nitrosamines have been a serious cause for concern. it has now become a requirement to accurately quantitate this group of compounds in pharmaceuticals during drug development and manufacturing. The formation of Nitrosamine Drug Substance Related Impurities (NDSRI) is also of concern during the API manufacturing process, and their presence presents additional separation challenges.

We have developed resources to aid you in the development of LC-MS quantitation.

Nitrosamines, Meeting Regulatory Requirements

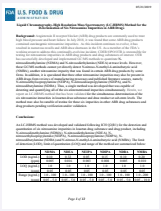
Enhanced sensitivity for nitrosamine analysis in a metformin active pharmaceutical ingredient (API)

13 nitrosamines were analysed in metformin using a Kinetex Biphenyl column and a SCIEX® 7500 instrument. This combination allows for LOQ levels as low as 0.01 ng/mL.



LC-HRMS Method for the Determination of Six Nitrosamine Impurities in Losartan Drug Substance or Drug Product

This workflow was developed and recommended by the USFDA for determination of six nitrosamine impurities in Losartan (and potentially other Sartan drugs). The column used is a Kinetex™ F5, which in combination with a HR-MS, provided LOQ < 1.0 ng/mL (0.05 ppm).



Low Level Quantification of 10 Mutagenic Nitrosamine Impurities in Acyclovir

In this technical note we show the selectivity and retention of Luna™ Omega Polar C18, which provides baseline resolution of 10 nitrosamines from acyclovir. Combining this with the SCIEX® QTRAP 6500+ allowed for linearity across the range 0.025-20 ng/mL.

LC-MS/MS Quantitative Analysis of NDMA in Ranitidine Active Pharmaceutical Ingredient (API) and Drug Product using the SCIEX 4500 QTRAP

This workflow is based on the analytical method developed and recommended by the USFDA. The column used is a Kinetex Biphenyl, which provides adequate retention and resolution of NDMA from ranitidine.

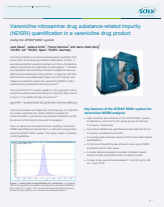
Read more about our Nitrosamines solutions.

Nitrosamines

Sensitive and Reproducible Quantification of N-Nitroso Propranolol in a Propranolol Drug Substance and Product

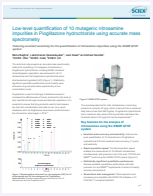
The formation of N-nitroso compounds can occur when molecules such as propranolol contain an amine group. It is possible for sodium nitrite to react with the amine under acidic conditions forming the N-Nitroso Propranolol impurity. Such NDSRI compounds may exhibit mutagenic potential greatly in excess of NDMA. In this technical note we show the use of Kinetex™ Biphenyl, which is able to retain N-Nitroso Propranolol away from any matrix interferences, allowing for a lower limit of quantification of 0.01 ng/mL.

Varenicline Nitrosamine Drug Substance-related Impurity (NDSRI) Quantification in a Varenicline Drug Product



Varenicline is a smoking cessation medication which has been found to form a nitrosamine related impurity. Due to the potential carcinogenic nature of such an impurity it is necessary to monitor for its presence. This technical note, which uses Kinetex Biphenyl as the analytical column, allows for a lower limit of quantitation of the varenicline NDSRI of 0.1 ng/ml. The method makes use of ion ratio values to increase specificity of the analysis in drug product matrix.

Low-level Quantification of 10 Mutagenic Nitrosamine Impurities in Pioglitazone Hydrochloride using Accurate Mass Spectrometry



Pioglitazone is a type 2 diabetes treatment, as such careful monitoring of nitrosamine contamination is required due to the likelihood of long-term prescription. In this technical note high resolution mass spectrometry is used to facilitate high sensitivity, separation is first performed using a Kinetex Biphenyl column, which allows for baseline resolution of the 10 target nitrosamines before the elution of pioglitazone, allowing for the API peak to be diverted to waste to avoid detector overloading and possible contamination.

Read more about our Nitrosamines solutions.

Application Handbook

Chromatography Solutions for API Manufacturers

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