Chromatography Solutions

Technical note #SP001

Enhanced LC Sample Preparation with J.T.Baker® High Performance Syringe Filters

INTRODUCTION

Many samples analysed by liquid chromatography (LC) contain particulates that can potentially damage both the LC instrumentation and the analytical column. Particulates can range from large particles and micro-organisms to fine, sub-micron suspended particles. Insufficient sample filtration can result in system blockages, increases in back pressure, reduced column lifetime and distortion of the analyte peak (e.g. increased peak tailing, split peaks etc). It is therefore important to remove particulates from the sample prior to analysis. Single use, disposable syringe filters provide a quick and convenient approach, the sample is drawn into a syringe and then pushed through an attached syringe filter into a sample vial, ready for LC analysis.

To ensure effective particulate removal and to preserve the integrity of the sample, it is essential to use high-quality syringe filters that provide reproducible and efficient filtration, and that maintain the sample integrity by not contaminating the sample by leaching extractable components during use. This Technical Note introduces the new range of syringe filters from J.T.Baker® and demonstrates how they provide exceptional performance to ensure confidence in the analytical data produced.

PREMIUM SYRINGE FILTERS

J.T.Baker® syringe filters are premium filters that have been specifically designed for the filtration of chromatography samples. They have been optimised to deliver the highest levels of performance and provide consistent and reproducible results with minimal extractables/leachables. These HPLC syringe filters are manufactured in ISO 9001 certified facilities to ensure the highest quality and ensure reproducible batch performance. They are available with a wide range of membranes (Table 1) and a range of filter diameters (13, 25 and 30 mm) and pore sizes (0.22, 0.45 and 1.00 µm) to suit every application. The housing is manufactured from polypropylene and the filters are batch tested and certified for housing burst pressure, bubble point, flow rate performance and extractables. J.T.Baker® syringe filters are also available with built-in glass fibre pre-filters

Table 1: Summary of membrane materials available, key features and target applications.

Membrane Material	Main Features	Applications
Nylon*	Hydrophilic Robust Broad chemical compatibility pH range: 3-14	Chemical filtration Beverage filtration HPLC sample preparation
PES*	Hydrophilic High asymmetry; high flow rate Low extractables Low protein binding pH range: 3-12	Protein filtration Buffer prep HPLC sample preparation
PTFE*	Hydrophobic Broad chemical compatibility pH range: 1-14	Organic solvents Strong acids and alkalis Gas filtration or air sampling HPLC sample preparation
H-PTFE*	Hydrophilic Broad chemical compatibility pH range: 1-14	Universal filtration Organic solvents Strong acid and alkaline resistance HPLC sample preparation
Regenerated Cellulose (RC)*	Broad chemical compatibility pH range: 3-12	Organic solvents General aqueous
Glass Fiber	Broad chemical compatibility High particulate capacity pH range: 3-14	Clarification and prefiltration DNA/RNA adsorption and purification

* Also available with glass fibre pre-filter for applications involving high particle-load samples

(double layer type, 100% binder-free borosilicate glass fibre), which is specially designed for high-particle loading and is an ideal solution for liquids that are difficult to filter.

WHY USE SYRINGE FILTERS?

If a sample contains particulate matter and is analysed by LC without filtration, the particulates may block the tubing within the LC system, or may accumulate at the head of the LC column. This can result in an increase in back pressure, distortion of peak shape, loss of performance and a reduction in column lifetime, ultimately compromising the analytical data generated. Figure 1 demonstrates the impact that effective sample filtration can have on the LC column. In this experiment a sample containing a 5% latex solution of polystyrene beads (0.46 – 1 µm) was injected onto an Avantor® ACE® Excel 3 C18 column (50 x 3.0 mm), with and without use of a syringe filter. When the sample is filtered using a 0.45 µm syringe filter prior to injection, the beads are successfully filtered from the sample and the column backpressure remains constant (103 ± 2.5 bar) for 500 successive injections. When the sample is not filtered, an identical column back pressure is initially obtained for the first 10 injections. However, the pressure then begins to slowly increase, as the particulates accumulate at the head of the column, before increasing dramatically after 50 injections, as the column is blocked and the backpressure exceeds the pressure limits of the LC column. This experiment demonstrates how the use of syringe filters can protect the column and dramatically extend the column lifetime.

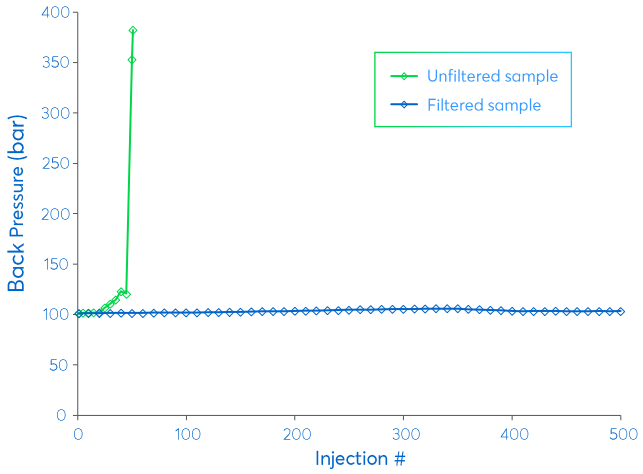


Figure 1: Plot of recorded column backpressure vs injection number for an experiment injecting a 5% latex solution of polystyrene beads (0.46 – 1 µm) onto a 3 µm C18 column using an LC system with a 400 bar pressure limit. The green trace shows the results obtained for injection of the sample without filtration, whilst the blue trace shows the results when the sample is filtered using a J.T.Baker® H-PTFE, 0.45 µm syringe filter prior to injection. Column: Avantor® ACE® Excel 3 C18 column (50 x 3.0 mm); Mobile phase: MeOH:H_2O (70:30 v/v); Flow rate: 0.43 mL/min; Temperature: 22 °C; Injection volume: 5 µL. The plot shows every 5th injection for the unfiltered sample and every 10th injection for the filtered sample.

J.T.BAKER® SYRINGE FILTERS FOR HIGH PERFORMANCE SAMPLE FILTRATION

To provide reliable protection for the LC instrumentation and column, it is important to use syringe filters with well-defined and reproducible porosity to ensure that particulates are always removed from samples. J.T.Baker® syringe filters are manufactured to strict standards to ensure efficient and reproducible extraction of particulates. Figure 2 shows experimentally determined extraction efficiencies for the range of 0.22 µm syringe filters. The data bars represent triplicate filters for multiple batches (n = 2-6). All membranes tested showed excellent extraction efficiencies, demonstrating their high performance and excellent reproducibility.

In addition to providing reliable particulate removal, it is also important that syringe filters do not release chemical components that may contaminate the sample. In LC analysis, the release of such extractable components can result in the presence of contaminant or "ghost" peaks in the resulting chromatogram. These peaks may interfere with the accurate identification and quantification of target analytes and potentially even be mis-identified as analytes of interest. Ultimately, the use of poor quality filters can severely compromise the analytical results obtained.

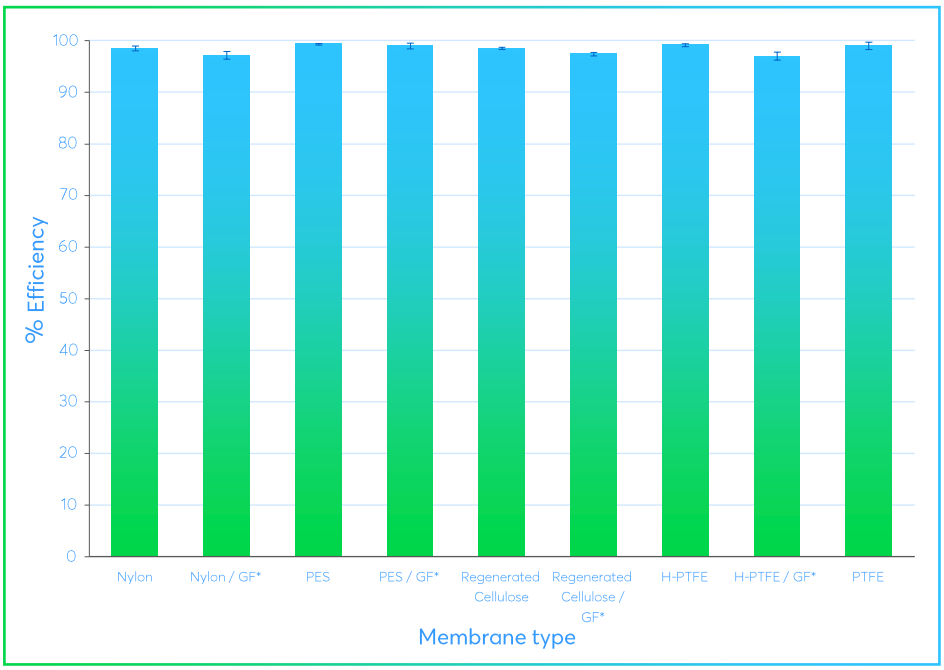
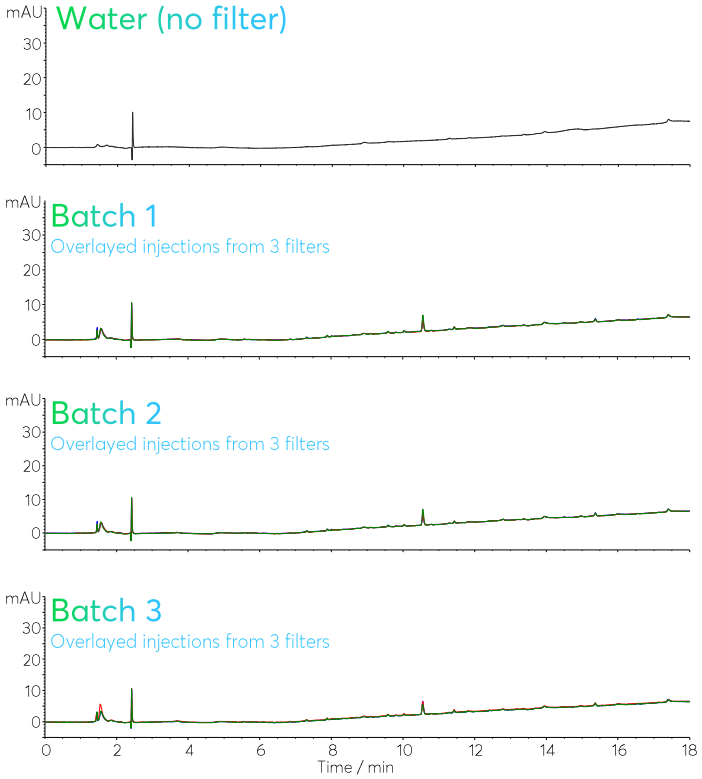


Figure 2: Extraction efficiency of J.T.Baker® 0.22 µm syringe filters by membrane type. Extraction efficiencies were experimentally determined by filtering a 0.01% latex solution of 0.3 µm polystyrene beads using syringe filters containing the various membranes, followed by determination of extraction efficiency against a set of prepared calibration standards by spectrophotometry (UV, 272 nm). Triplicate analyses were performed for multiple batches. Samples were prepared in water for hydrophilic membranes and in methanol for hydrophobic membranes. *Refers to filters with built-in glass fibre pre-filters.

The high performance materials used to manufacture J.T.Baker® syringe filters minimises the potential for leaching of extractable components, thereby ensuring the integrity of filtered samples is maintained. Figure 3 shows the chromatograms obtained from an extractables testing procedure carried out on 3 batches of 0.22 µm J.T.Baker® H-PTFE filters. In this test, 1.5 mL of water was filtered using the H-PTFE filter and 100 µL of the resulting eluent injected onto an LC column and analysed by gradient elution. The injection volume used is much greater than typically used for LC analysis on this column dimension to ensure that this is a demanding test, capable of detecting trace-level components extracted from the filters. All three batches showed consistently low-levels of detectable extractable components, demonstrating the high performance of these filters.

To further demonstrate the importance of using high quality syringe filters for LC sample preparation, the performance of J.T.Baker® filters were compared to a competitor filter for the LC analysis of nitrosamines in a pharmaceutical active ingredient (API). Nitrosamines are highly genotoxic compounds that require trace-level determination to ensure the safety of pharmaceutical products, often by LC-MS/MS. In this example, a 67 mg/mL sample of valsartan was spiked with eight nitrosamines at 1 µg/mL. The sample preparation involves extraction in 1% formic acid to precipitate the valsartan, therefore the samples were filtered using 0.45 µm nylon syringe filters prior to LC analysis. The competitor filter was found to contaminate the sample with several extractable components that could interfere with the analysis. An unknown component partially coelutes with peak 4 (NEIPA), compromising accurate integration, whilst another component elutes between peaks 6 (NMPA) and 7 (NDPA), completely obscuring peak 6 and preventing accurate integration and quantification. A major contaminant was also observed to elute at 3.8 minutes. The chromatograms obtained from the two samples clearly demonstrates the superior performance of the J.T.Baker® syringe filters.



Column:	Avantor® ACE® Excel 3 C18 150 x 4.6 mm
Mobile phase	A: H_2O B: MeCN
Flow rate:	1 mL/min
Injection volume:	100 µL
Temperature:	30 °C
Detection:	UV, 214 nm
Gradient:	

Time (mins)	%B
0	5
15	100
20	100
21	5

Sample:	1.5 mL water filtered through J.T. Baker® 0.22 µm H-PTFE syringe filters. Three different batches were tested (three filters tested from each batch).

Figure 3: Chromatograms showing results of an extractables test performed on three batches of J.T.Baker® 0.22 µm H-PTFE syringe filters. Three filters were tested from each batch.

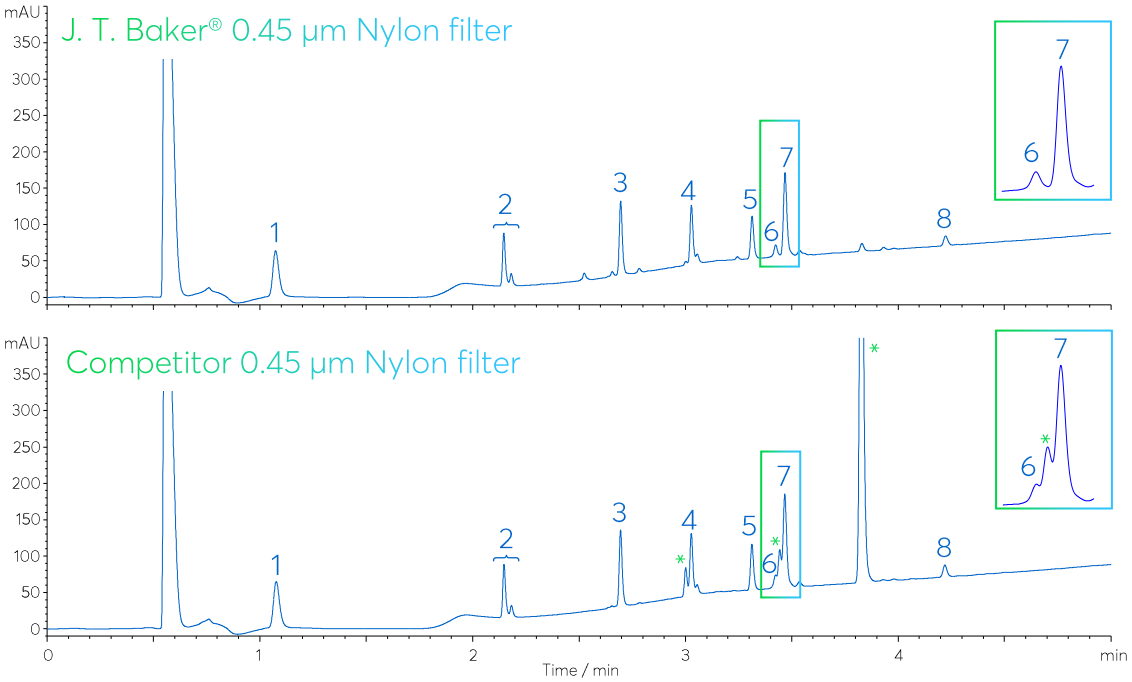


Figure 4: Comparison of chromatograms obtained from filtering a sample of valsartan spiked with eight nitrosamines with J.T.Baker® and competitor syringe filters..

CONCLUSION

Liquid chromatography columns and instrumentation can be damaged by particulate material originating from injected samples. It is therefore important to adequately filter samples to extend column lifetime and protect the LC instrumentation. J.T.Baker® Syringe filters provide a convenient, high performance solution to achieve this. The use of premium quality syringe filters is highly recommended to ensure that valuable samples are filtered efficiently, reproducibly and without introducing extractable components that could contaminate valuable samples and compromise the analytical results obtained. The data and applications outlined in this short article have demonstrated the high performance that can be expected from J.T.Baker® premium syringe filters and their suitability for use in demanding applications.

Column:	Avantor® ACE® Excel 2 C18, 100 x 2.1 mm
Mobile phase	A: 0.1% formic acid in MeOH:H_2O 98:2 v/v B: 0.1% formic acid in MeOH:H_2O 2:98 v/v
Flow rate:	0.5 mL/min
Injection volume:	20 µL
Temperature:	40 °C
Detection:	UV, 240 nm
Gradient:	

Time (mins)	%B
0	0
1	0
2.5	50
5	86.5
5.5	86.5
5.6	0

Sample:	Nitrosamines spiked into 67 mg valsartan at 1 µg/mL, then filtered through 0.45 µm nylon syringe filter 1. NDMA, 2. NMBA, 3. NDEA, 4. NEIPA 5. NDIPA, 6. NMPA, 7. NDPA, 8. NDBA *Contaminant peak

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