Chromatography Solutions

Assessing Syringe Filter Performance for Liquid Chromatography Samples

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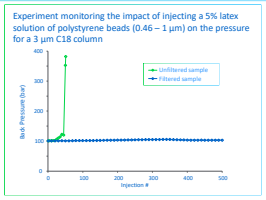
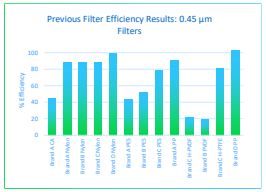
1. Background

- Particulates in LC samples may potentially damage LC instrumentation and the analytical column resulting in:
  - System blockages.
  - Back pressure increase.
  - Reduced column lifetime.
  - Peak distortion.
- Particulates can range from large particles/ microorganisms to sub-micron suspended particles.
- Single use syringe filters provide a quick and convenient approach to particulate removal.
- Use of poor quality syringe filters can lead to:
  - Insufficient particle removal.
  - Contamination from extractable components.
  - Poor reproducibility.
- Previous work in our lab found filter quality can be highly variable.
- This work summarises data from two simple tests used to characterise a range of J.T.Baker® syringe filters.





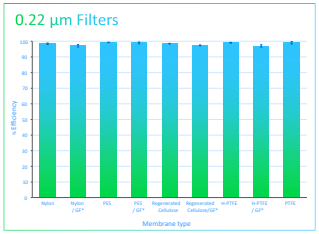
2. Extraction Efficiency Test

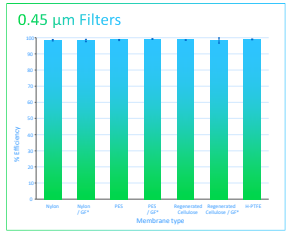
- The extraction efficiency was tested by determining the amount of latex beads removed from a solution.
- Method:
  - 0.01% Latex bead (polystyrene) solutions made up in H_2O (hydrophilic filters) or MeOH (hydrophobic filters).

Filter porosity (µm)	Latex bead size (µm)
0.22	0.3
0.45	0.46
1.0	1.1

  - 1 mL of suspension filtered.
  - Eluent analysed using a VWR UV-3100PC spectrophotometer.
  - Detection: UV, 272 nm.
  - Spectrophotometer calibrated between 0.001 and 0.01 % (6-point).

- Example Data: J.T.Baker® syringe filters with different membrane materials tested.
- 0.22 and 0.45 µm filters, 3 filters from multiple batches (n = 2-6) tested.
- Excellent extraction efficiency demonstrated.
- Excellent reproducibility.
- 0.45 µm equivalents showed similar extraction efficiency.

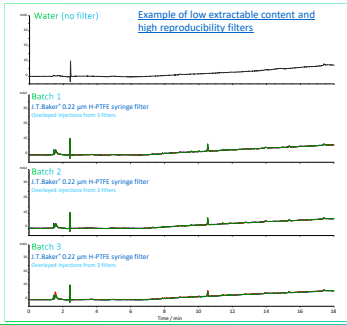


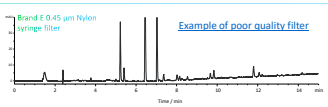


* GF denotes filters with glass fibre pre-filter for applications involving high particle-load samples

3. Leachable/Extractables Test

- There is potential for extractables and leachables to come from the filter (membrane or housing) during use.
- Extractable components may appear as contaminant or ghost peaks during LC analysis.
  - Potential to interfere with identification/quantification of target analytes.
- Filters can be easily tested for extractables by LC-UV.
- Use of high quality syringe filters will protect sample integrity.

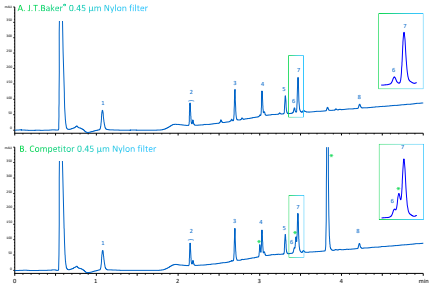




- Method:
  - 1 mL of H_2O or MeOH passed through filter.
  - Eluent analysed by HPLC:
  - Column: Avantor® ACE® 3 C18, 150 x 4.6 mm
    - Mobile phase A: H_2O
    - Mobile phase B: MeCN
    - Gradient: 5 to 100% B in 15 mins.
    - Flow rate: 1 mL/min
    - Temperature: 30 °C
    - Detection: UV, 214 nm
    - Injection volume: 100 µL

4. Example Application: Nitrosamines

- Performance comparison of J.T.Baker® Nylon syringe filter and equivalent.
- Trace analysis of genotoxic impurities in pharmaceutical API.
- Performance of syringe filter is therefore critical in preparation of samples.
- Contaminant peaks extracted from the filter in B prevent accurate determination of NEIPA (4), NMPA (6) and NDPA (7).
- Major contaminant peak also elutes at 3.8 minutes in B.



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
Inj. 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

5. Summary and Conclusions

- Syringe filters are commonly used in sample preparation for LC analyses.
- Filter quality can vary markedly.
- Poor filter efficiency leads to failure to remove particulates from the system.
  - Potential damage to LC system and column.
- Poor quality filters may leach extractable components.
  - Interference with target analyte identification and quantification.
- Filter performance can be easily assessed.
- Filter efficiency can be tested by filtering suspension of latex beads, followed by quantitative testing using spectrophotometry.
- Filters can be tested for presence of leachables and extractables by eluting with a suitable solvent and eluent analysed by LC.