Protein precipitation (PPT) protocol for Mini-UniPrep™ Syringeless filters

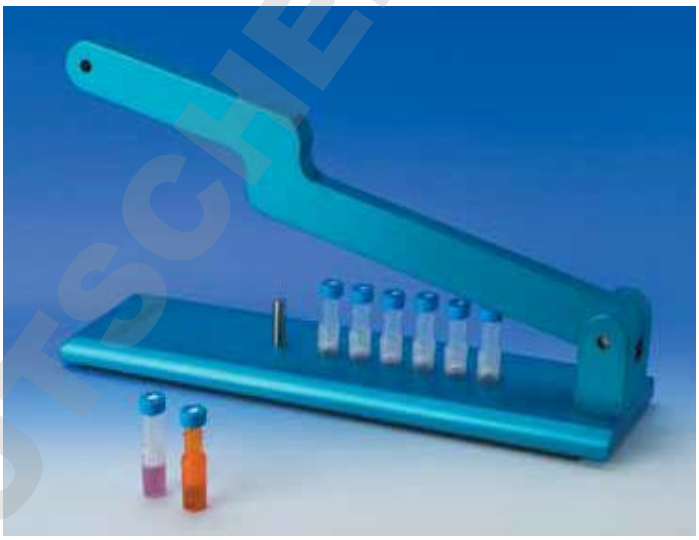
Whatman Mini-UniPrep Syringeless Filters offer a highly efficient, simple alternative for removing unwanted protein prior to HPLC/MS analysis. Ideal for performing analytical characterization in drug research, the method utilizes acetonitrile precipitation and filtration by compression to remove protein from plasma, serum, whole blood, and other biological fluids. It is a single tube method that saves time and eliminates the manual transfer steps which make spin clarification problematic.

Fast, convenient all-in-one filtration for HPLC sample prep

- **Simple, one-step, single-tube method:** Eliminates centrifugation plus aspiration issues and transfer steps.
- **Process samples 3 times faster:** Purify six samples in three minutes, 48 samples in < 30 minutes.
- **> 99% protein removal for plasma samples:** Enables compound quantitation and extends column life.
- **Self-sealing septa allows for repeated injections:** Prevents evaporation and maintains sample integrity.
- **Compatible with all major autosamplers**

Mini-UniPrep Compressor

The Mini-UniPrep Compressor enables up to six Mini-UniPrep filter devices to be processed simultaneously. This handy tool will speed sample prep processing and reduce the risk of carpal tunnel syndrome.



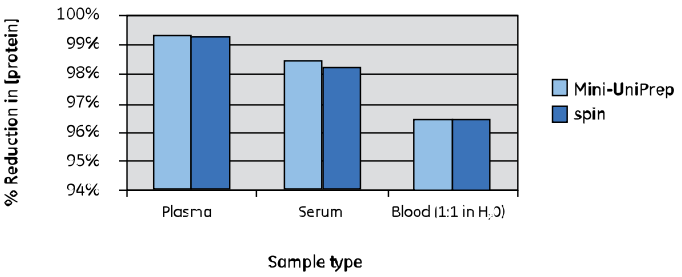


Fig 1. Efficient protein removal for different sample types

The effectiveness of removing protein from different sample types was evaluated. Plasma, serum, and whole blood (diluted 1:1 in H_20) were run through the Mini-UniPrep-PPT procedure. The same samples were also run through a conventional spin methodology which utilizes a 1.5 ml microfuge tube in place of the Mini-UniPrep, and a five minute centrifuge at 10 000 x g to bring the proteins down. Supernatant was aspirated into a separate tube for analysis. Micro-BCA was used as previously described to quantitate proteins. All results are reported in percentage reduction in protein concentration.

Mini-UniPrep™ Protein Precipitation Protocol

1. Add 400 µl acetonitrile to Mini-UniPrep sample chamber
2. Add 100 µl plasma to Mini-UniPrep sample chamber
3. Insert Mini-UniPrep filter plunger until first locking ring is engaged
4. Vortex sample mixture for 3 to 5 s
5. Place in compressor unit and compress until second lock ring is engaged
6. Analyze sample

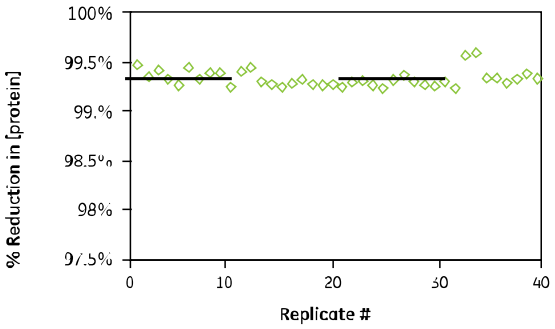


Fig 2. Reproducibility of protein removal

The reproducibility of protein removal from plasma samples was measured by applying the Mini-UniPrep-PPT procedure to 40 replicates of normal human plasma. Filtrate was removed from the Mini-UniPrep and dried down in a microfuge tube prior to saline resuspension and micro-BCA protein quantitation. Percent protein removal was calculated by comparing the protein concentrations of pre- and post-filtration plasma/Acn mixtures.

Table 1. Ordering information

Catalog no.	Pore size (µm)/membrane	Qty/Box
UN203NPUORG	0.45 PTFE	100
UN503NPUORG	0.45 PTFE	1000

Catalog no.	Description	Quantity
CR0000006	Six position Compressor	1

www.gelifesciences.com/whatman

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imagination at work

Table 2. Productivity advantages versus spin method

	Mini-UniPrep-PPT	Spin method
Dead volume	50 µl	Variable
Single sample processing time (plasma tube → HPLC)	~ 30 s • add sample • add acetonitrile • insert plunger • vortex • compress plunger • place in autosampler	~ 6 min • add sample • add acetonitrile • close cap • vortex • centrifuge • aspirate supernatant to chromatography vial • close vial screw cap • place in autosampler
6 sample processing time	~ 3 min	~ 10 min
48 sample processing time	~ 30 min	~ 45 min (> 45 min if centrifuge capacity is < 48 samples)
Disposable required per sample	1 pipette tip	2 pipette tips 1 microfuge tube 1 chromatography vial
Additional accessories	Six position compressor	Microcentrifuge
Comments	Filtered sample cannot be dried down to exchange solvent	Time consuming supernatant aspiration is problematic

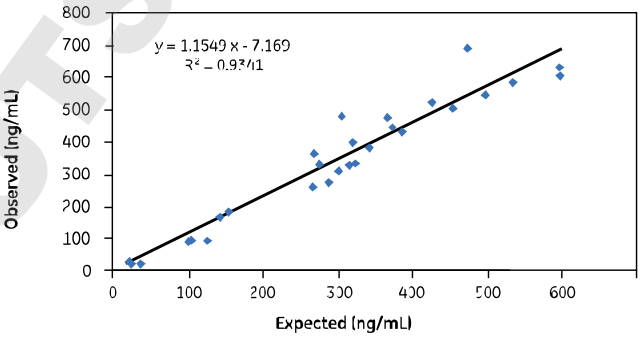

Fig 3. HPLC quantitation of caffeine in plasma

Plasma samples containing varying amounts of caffeine were prepared in blinded fashion and processed using the Mini-UniPrep along with known plasma standards containing caffeine. After compression filtration, the standards and "unknowns" were analyzed by HPLC using 10% ACN/90% H_20 mobile phase and a Partisil™ 5-ODS-3 reverse phase column (4.6 x 100 mm) at a flow rate of 2 ml/min. The OD_{275} nm caffeine peaks as measured used to determine the caffeine concentration of the unknown samples by comparison to the standard set.

For more information on Mini-UniPrep Syringeless filters, or any other Whatman products visit our web site at **www.whatman.com**

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