Tip-on-Tip™ Protein Precipitation

HIGHLIGHTS: High Reproducibility, High Throughput, Seamless Integration

ToT

PURPOSE AND OBJECTIVE

- Introduce innovative, automated multi-purpose INTip™ solution as an alternative to centrifugation utilizing a patent pending Tip-on-Tip (ToT) technology.
- Demonstrate the feasibility of a novel and rapid protein precipitation method, often referred to as "protein crash".
- Develop automated methodology to minimize laborious manual steps such as vortex mixing and centrifugation and maximize throughput.
- This method can be extended to many areas of science, including drug discovery, metabolomics, and clinical trial studies.
- This automated procedure could be applied to applications such as gelatin removal from drug formulations or processing cell lysates.

METHOD

Traditional Manual Workflow

Add ACN

↓

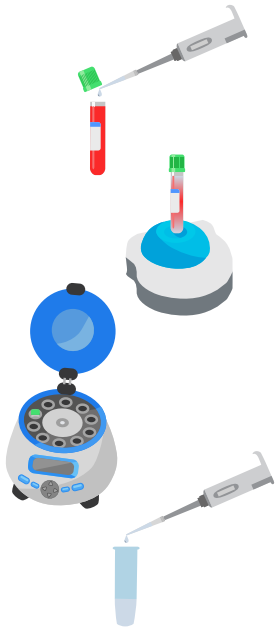
Vortex

↓

Centrifuge

↓

Transfer Supernatant



> 15 minutes

Automated Tip-on-Tip Workflow

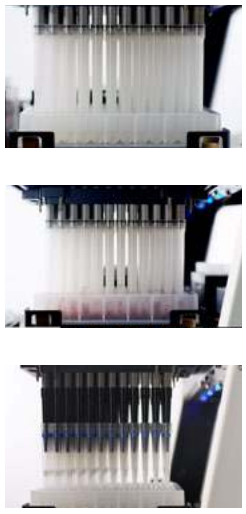
Add ACN

↓

Mix via aspirate/ dispense

↓

Tip-on-Tip



< 5 minutes/96 samples

Traditional protein precipitation techniques involve manually pipetting acetonitrile into tubes of sample, vortex mixing, and then centrifuging (represented above). This process generally takes at least 15 minutes to complete. Further, this methodology does not readily allow for multiple samples to be processed in tandem. ToT technology offers a protein precipitation workflow that will protein precipitate and filter up to 96 samples in under five minutes. This ToT method can greatly improve throughput by orders of magnitude.

RESULTS AND DISCUSSION

The response for 32 common therapeutic and abused drugs were generally found to be similar with the ToT method, suggesting that the protein precipitation (or "crash") is just as efficient as vortex mixing. On average, the peak intensities from the ToT method were 20-40% higher. The increased intensities are most likely a result of being able to obtain all filtrate in the ToT method versus the limitation of pipetting the supernatant with centrifugation. Full mass spectral scans of the extracts using ToT and centrifugation appear to be identical (data not shown).

In addition to whole blood, ToT protein precipitation methodology can also efficiently process serum samples. Approximately 90% of 25-hydroxyvitamin D is protein bound. Therefore, thorough protein precipitation is paramount to release the analyte for accurate analysis. Once again, ToT was compared to traditional protein precipitation using real human serum samples. The quantitative comparison produced an average percent difference of 2%. The chromatograms (right) show the improved signal-to-noise when using ToT. For more information regarding DPX solutions for Vitamin D, please see the application note.

In addition to providing a viable on-line alternative to centrifugation, ToT can be easily complimented by additional INTip solid phase extraction when necessary.

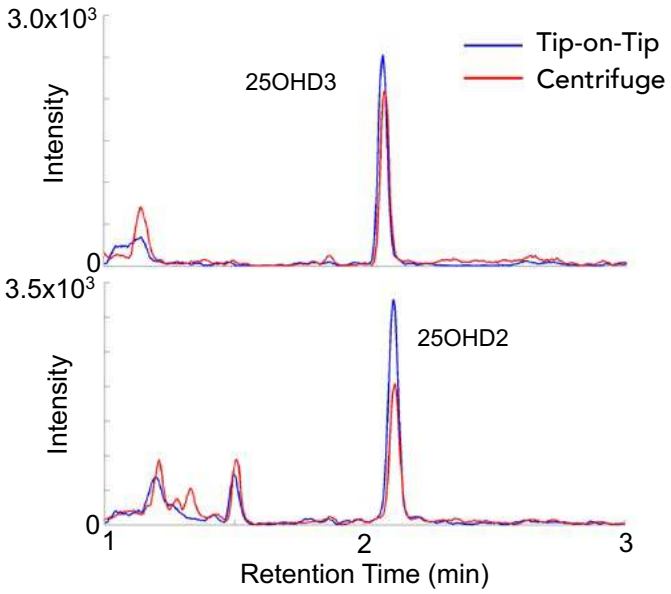


Figure 2. The chromatographic comparison of the DPX Tip-on-Tip method and traditional centrifiguation results using patient samples.

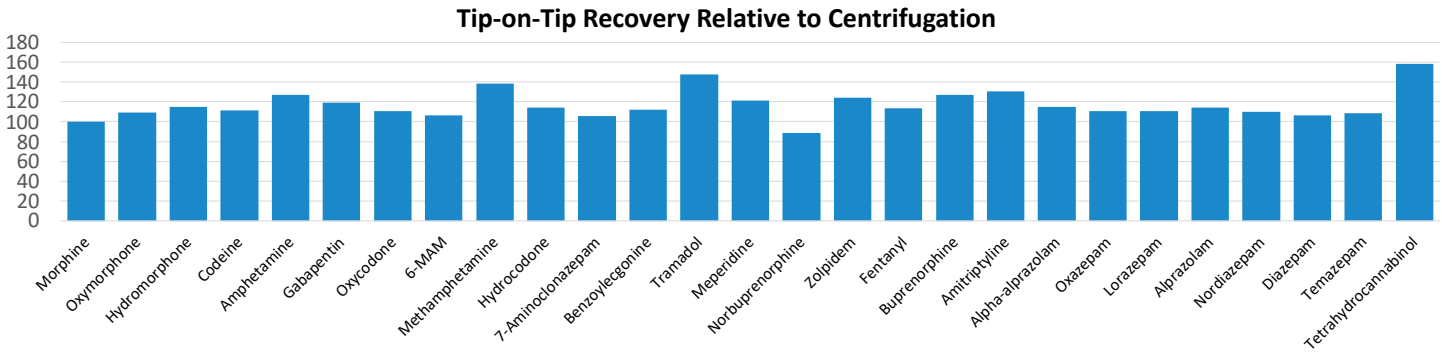


Figure 3. These studies show that efficient protein precipitation using an automated liquid handler with simple pipetting commands can be substituted, in most cases, for laborious vortex mixing and centrifugation. The ToT filtering of the sample solution provided higher responses than the centrifuged samples. ToT has the capability to increase throughput by allowing up to 96 samples to be protein precipitated and filtered in less than 5 minutes.

FILTRATION TIP PRODUCT INFORMATION:

Low Porosity Tips

High efficiency sub-micron matrix filtration including urine, blood, and serum.

Single Phase Tips

Minimal dead volume ideal for small volume blood and serum protein precipitation applications.

Dual Phase Tips

Two filters in series provide maximum filtration without clogging for higher volume blood and serum protein precipitation applications.

CUSTOM WORKFLOW SOLUTIONS

Our team of application scientists supports custom method development to help you seamlessly integrate our products into your work-flow.

info@dpxlabs.com

dpxtechnologies.com