

Fast and Effective Complex Sample Desalting for LC/MS/MS Analysis Using the Thermo Scientific Aspire RP30 Desalting Tips

Catherine CL Wong, Ph.D.

Department of Chemical Physiology, The Scripps Research Institute, La Jolla, San Diego, CA.

Key Words

- Aspire
- Sample Desalting
- Peptide Desalting
- Peptide Clean-up
- LC/MS
- LC/MS/MS

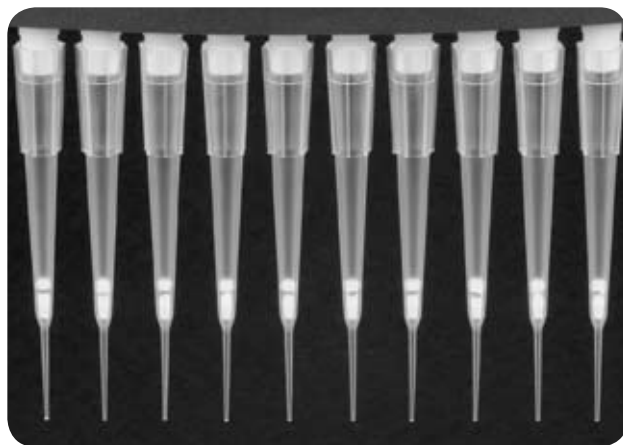
Introduction

Mass Spectrometry (MS) has become a powerful tool for the analysis of complex protein samples. Shotgun or bottom-up proteomics requires the analysis of thousands of peptides generated by an enzymatic digestion which can be achieved by using LC/MS/MS methods.¹ Highly complex samples often contain salts, detergents and other ion-suppressing contaminants that limit the ionization process resulting in lower peak intensities and compromised peptide/protein detection. It is therefore essential to remove this interference from the samples before subjecting them to MS analysis.

Here we report a fast and effective way to facilitate MS sample cleanup. Two desalting methods, the Thermo Scientific Aspire RP30 Desalting Tips and the traditional column flushing procedure, were employed to desalt a tryptic-digested yeast whole cell lysate (soluble fraction) prior to LC/MS/MS runs. By performing this side-by-side comparison, we observed an 11% increase in total identified proteins, 26% increase in total identified peptides and 50% increase in total numbers of spectra with the sample desalted by the Aspire™ tips. Due to the reduction in ion-suppression effects, sequence coverage was also improved with the sample subjected to the Aspire tip cleanup. In addition, the proprietary Styrene-divinylbenzene resin contained within the Aspire tips allows higher peptide recovery, which is better suited for desalting of complex samples. Overall, the 15-minute Aspire desalting protocol is fast and effective compared to the traditional 1 to 4-hour column flushing method.

Materials

1. Thermo Scientific Aspire RP30 Desalting Tips
2. Thermo Scientific Finnpiptette Novus 12-Channel Electronic Pipette, 30-300 µl
3. Tryptic-digested yeast whole cell lysate (soluble fraction)
4. Ultrapure water
5. Acetonitrile (ACN)
6. Trifluoroacetic Acid (TFA)
7. Methanol
8. Formic Acid (FA)
9. Activation Solution: 50% Methanol



10. Equilibration/Washing Solution: 0.5% TFA in 5% ACN
11. Sample Solution: 2% TFA in 20% ACN
12. Elution Solution: 70% ACN
13. Buffer A: 5% ACN, 0.5% FA
14. Buffer B: 80% ACN, 0.5% FA
15. Buffer C: 500 mM Ammonium Acetate, 0.5% FA

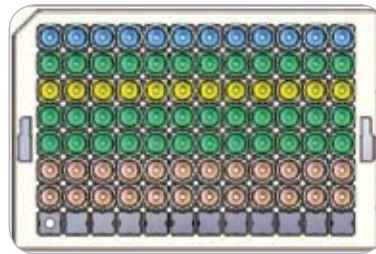
Method

One hundred micrograms of yeast whole cell lysate (soluble fraction) were subjected to tryptic digestion. One 50 µg fraction of the tryptic-digested peptide mixtures was desalted using two Aspire RP30 Desalting Tips, following the manufacturer's purification protocol. The Aspire protocol was carried out using a Finnpiptette® Novus 12-channel electronic pipette and the purification steps are illustrated in Figure A. The samples were pooled, dried, resuspended in Buffer A, and subsequently loaded onto a Multidimensional Protein Identification Technology^{2,3} (MudPIT) column for LC/MS/MS analysis. The remaining 50 µg of the tryptic-digested peptide mixture were desalted on a MudPIT column by flushing with Buffer A for 2-4 hours using an LC pump, and then subjected to the MS.

PREPARE TUBE RACK

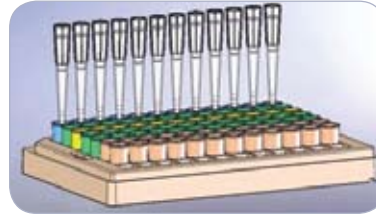
Aliquoted:

- ◆ 100 µl of Activation Solution to blue tubes
- ◆ 100 µl of Equilibrium/Washing Solution to green tubes
- ◆ Diluted sample 1:3 in Sample Solution
- ◆ 25 µl elution solution to orange tubes



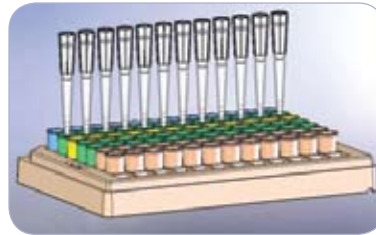
ACTIVATE (Row A, blue tubes)

Aspirated and dispensed Activation Solution 2X.



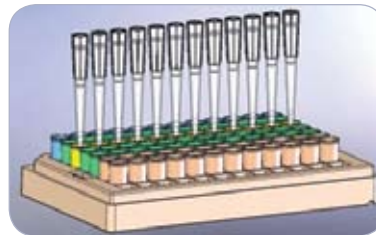
EQUILIBRATE (Row B, green tubes)

Equilibrated the resin by aspirating and dispensing equilibration/wash solution 2X.



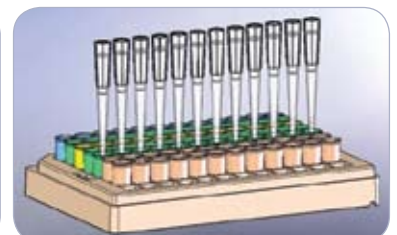
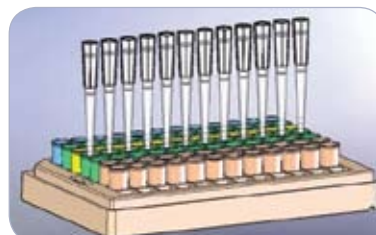
BIND (Row C, yellow tubes)

Aspirated and dispensed the sample fraction for 6X.



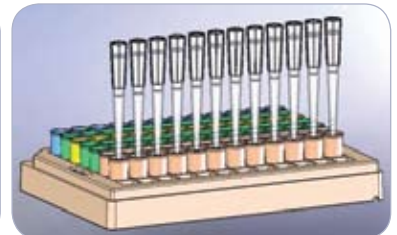
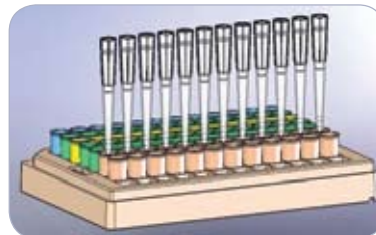
WASH (Row D and E, green tubes)

Aspirated and dispensed wash fraction one 2X. Repeated with wash fraction two.



ELUTE (Row F and G, orange tubes)

Aspirated and dispensed elution fraction one 5X. Repeated with elution fraction two. Pool fractions.



DRY and RESUSPEND

Placed entire orange elution collection tube inside of a 1.5 ml microcentrifuge tube. Proceeded with drying in a vacuum evaporator. Resuspend in 50 µl of Buffer A.



LC/MS/MS analysis

Figure A: The Aspire RP30 Desalting Tips Procedure

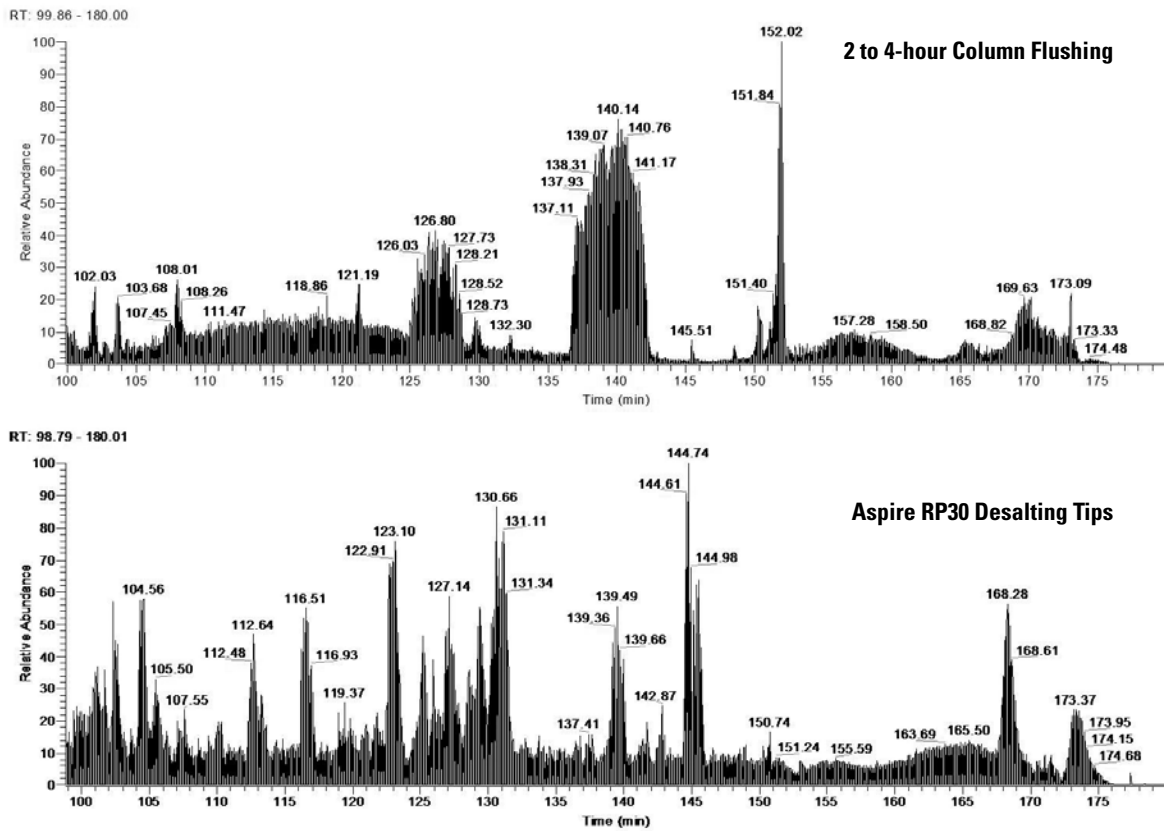


Figure B-1: First step reversed-phase gradient.

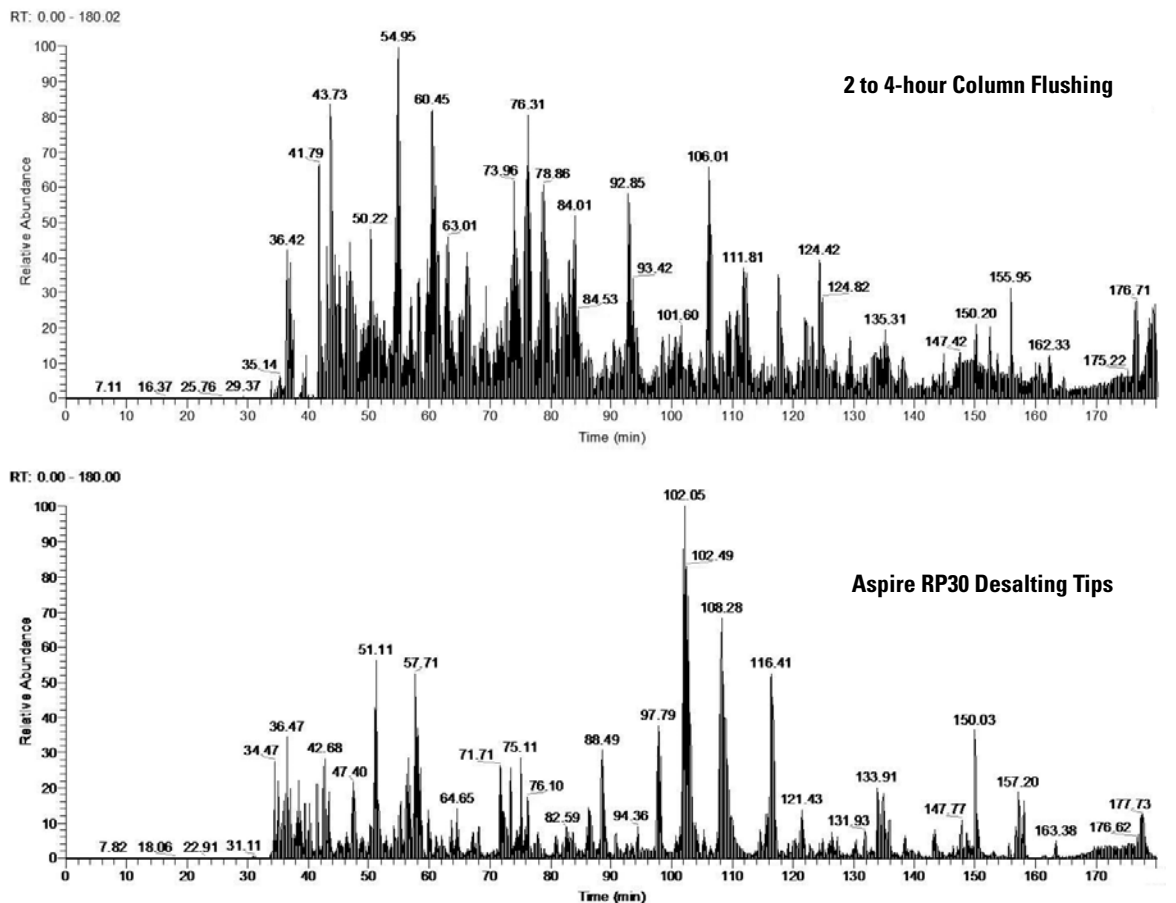


Figure B-2. Third step 40% Buffer C.

Figure B. Chromatograms of the two complex mixtures subjected to two different sample cleanup methods prior to LC/MS/MS analysis. The sample purified with the Aspire RP30 Desalting Tips shows increased signal-to-noise ratios and minimized ion suppression effects.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

	2 to 4-Hour Column Flushing			Aspire RP30 Desalting Tips		
	Proteins IDs	Peptides IDs	Spectra	Proteins ID	Peptides ID	Spectra
Unfiltered	11871	390025	416293	11879	677317	725580
Filtered	750	5780	33299	833	7290	50234
Forward matches	727	5734	33252	809	7239	50134

Table 1. Protein and peptide identification results of the 2-4 hour column flushing method compared to the Aspire RP30 Desalting Tips. The number of total proteins, peptides and spectra identified increase by about 11%, 26% and 50% respectively. The Aspire tips offer better peptide binding and recovery.

Results and Discussion

The desalting effects of the tryptic-digested yeast whole cell lysate using the Aspire RP30 Desalting Tips and the conventional column flushing method were compared. The sample subjected to the Aspire tip cleanup demonstrated greatly improved peptide recovery (Figure B and Table 1) compared to the traditional column flushing method. The Aspire tip desalting procedure was also substantially faster, saving 1-3 hours per sample processed.

The LC chromatograms of both desalted samples from the first step of reversed-phase gradient (Figure B1) and the 3rd step which is containing 40% Buffer C (Figure B2) are shown in Figure B. The Aspire RP30 Desalting Tips removed interference from the peptide mixture, significantly increased the peak intensity and signal-to-noise ratio, and minimized ion-suppression effects. The data shown in Figure B is a representation of two of the seven steps of the entire MudPIT experiment.

The protein and peptide identification results of the Aspire tips compared to the column desalting method are presented in Table 1. The sample subjected to the Aspire tip cleanup yielded significant improvements in peptide recovery, with an approximate 11% increase in total identified proteins, 26% increase in total identified peptides and 50% increase in total numbers of spectra. Sequence coverage was significantly improved with the Aspire tip processed sample (data not shown).

Conclusions

The Aspire RP30 Desalting Tips contain proprietary Styrene-divinylbenzene resin that effectively removes salts and other ion-suppressing interference from highly complex mixtures and are better suited for LC/MS sample cleanup. The Aspire tips offer greater peptide binding and recovery, increasing the numbers of total identified peptide, protein and spectra. Sequence coverage was also improved as a result of minimizing ion-suppression effects. In addition, the Aspire tip's rapid multichannel, color-coded purification protocol substantially reduces sample processing time, rendering it more efficient compared to the traditional time-consuming column flushing method.

References

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North America

+1 800 995 2787
mbp.info@thermofisher.com

Europe

+44 (0) 161 486 2110
mbp.info@thermofisher.com

Asia

mbp.info@thermofisher.com

www.thermo.com

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