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AttractSPE®Disks Tips for an Efficient protein cleanup Comparison with competitor products

Application Note AttractSPE®TipsC18

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In this application note, performances of AttractSPE®Disks Tips C18 and two marketed SPE Tips C18 are compared for the peptidic purification. As a model, the standard protein (BSA, Bovin Serum Albumin) was digested with trypsin and the peptides were then desalted and concentrated with SPE Tips C18. Peptides were finally analysed by nanoLC-MS/MS.
Generated data show excellent performance of the AttractSPE®Disks Tips C18 similar or even better than both marketed SPE tips and make possible an easy and reliable identification of the proteins.

AttractSPE®Disks Tips are Stage tips based on small particles densely packed and embedded in a soft membrane. This SPE disk makes possible the use of micro-elution volumes, eliminates the need for frits reducing dead volumes and gives excellent recovery. It is suitable for a wide range of domains like protein and peptide purification, fractionation/desalting, or drug analysis.

PROTOCOL FOR PEPTIDE PURIFICATION

10µg Bovine Serum Albumin (BSA) has been digested by Trypsin and then desalted as described below to analyze the peptides. During the SPE
process with one SPE tips C18, two fractions are collected for analysis. The fraction of peptides which passed through during the loading (NR or Not Retained) and the fraction resulting of the elution (R or Retained fraction). The analysis of peptides is carried out by a nanoLC-MS/MS. Testings are made in triplicate.
The same protocol is used for AttractSPE®Disks Tips C18 and two marketed SPE tips C18 (competitor 1 (ZipTip®) and competitor 2 (Omix)) to compare performances.
Abbreviations:
ACN: Acetonitrile
FA 0.1%: Formic acid solution at 0.1%

SPE METHOD USING SPE TIPS C18

1

1-Conditioning/Equilibration

1- 3x 10µl ACN/FA 0.1 % (50/50)
2- 3x 10µl FA 0.1%

***Centrifugation 2000rpm/2min after each washing step**

2

2- Loading of peptides

1- Change microtube
2- Load 10µL of sample (10µg of protein digest)
3- Wash with 3x 10µl FA 0.1%
4- Keep all not-retained fractions (NR - Not Retained)

***Centrifugation 2000rpm/2min after each washing step**

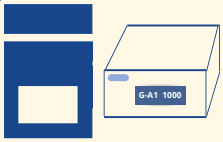
3

3- Elution

1- Change microtube
2- Elute with 2x10µL of ACN/FA 0.1% (50/50)
3- Elute with 2x10µL of ACN/FA 0.1% (80/20)
4- Keep all obtained fractions (Retained)

***Centrifugation 4000rpm/2min after each washing step**

4



4- LC/MS analysis

1- Dry sample with speed vacuum
2- Solubilize by adding 10µl FA 0.1% for MS analysis
3- Inject 1µl of sample diluted 1 to 5 to MS

RESULTS
- PROTEIN SEQUENCE COVERAGE

Table 1. Protein sequence coverage

Products	AttractSPE®Disks Tips		Competitor 1		Competitor 2	
Fraction	Retained	Not Retained	Retained	Not Retained	Retained	Not Retained
% coverage	75±2%	63,0±0,2%	69±3%	72±1%	75±3%	71±4%
PSM (Peptide Spectrum Matches)	161±9	70±4	125±19	167±9	146±6	166±15

AttractSPE®Disks Tips shows the higher number of Peptide Spectrum Matches (PSM) for the Retained fraction (161 compared to 125 and 146). AttractSPE®Disks Tips supplies more information than competitors 1 and 2 for the identification of the proteins. This is very important to identify complex mixture.

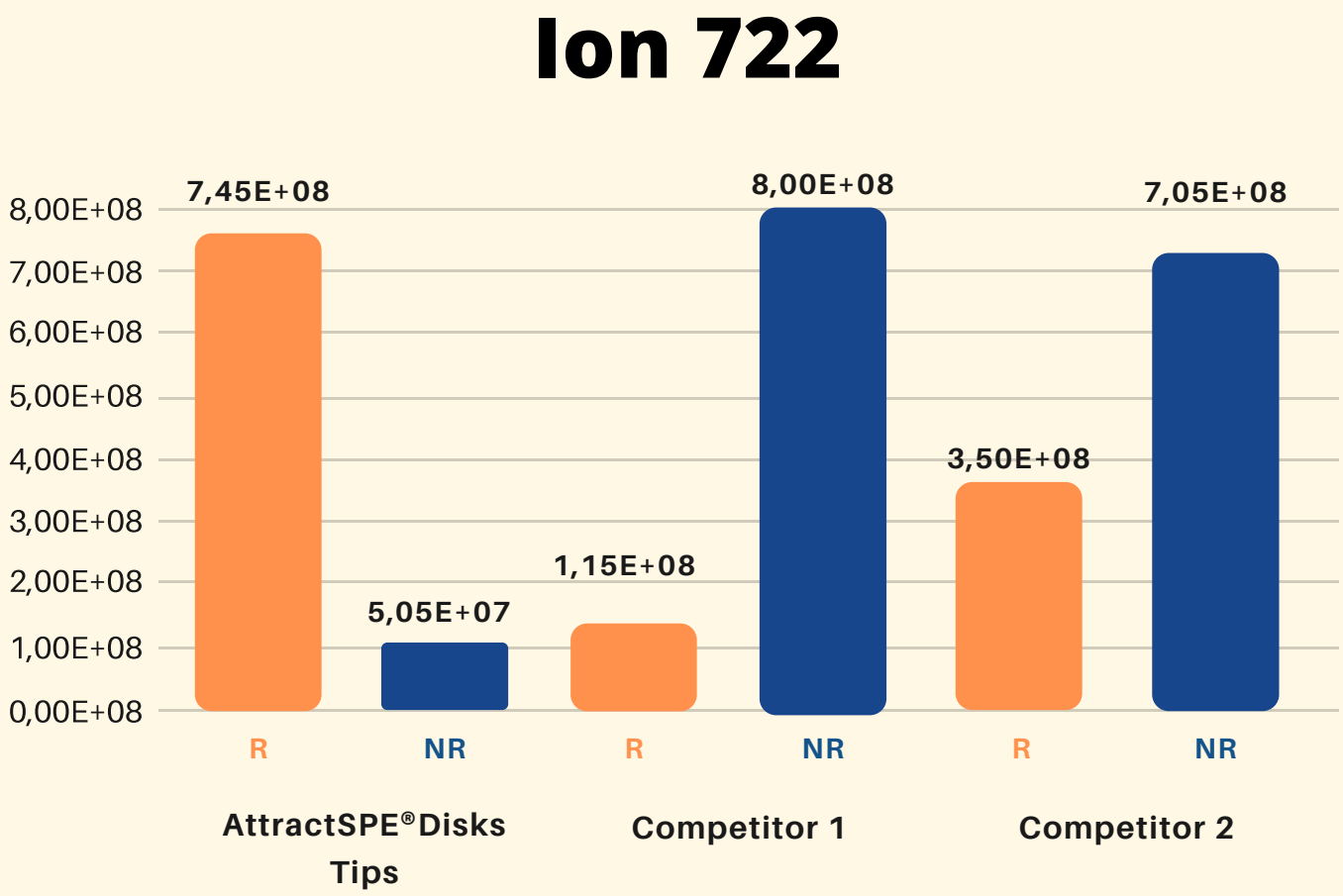
In addition, a percentage of coverage for the retained fraction of 75% was obtained for AttractSPE®Disks Tips and competitor 2. This is very important and an excellent result for the identification of proteins. This indicates a very good retention of peptides by the disks.

In the not-retained fraction, the %coverage and PSM of AttractSPE®Disks Tips were the lowest, reducing the loss of information.

With these parameters, an easy and reliable identification of the digested proteins can be done with AttractSPE®Disks Tips C18.

COMPARISON OF IONS INTENSITIES

Graph 1. Ions intensities for Ion 722



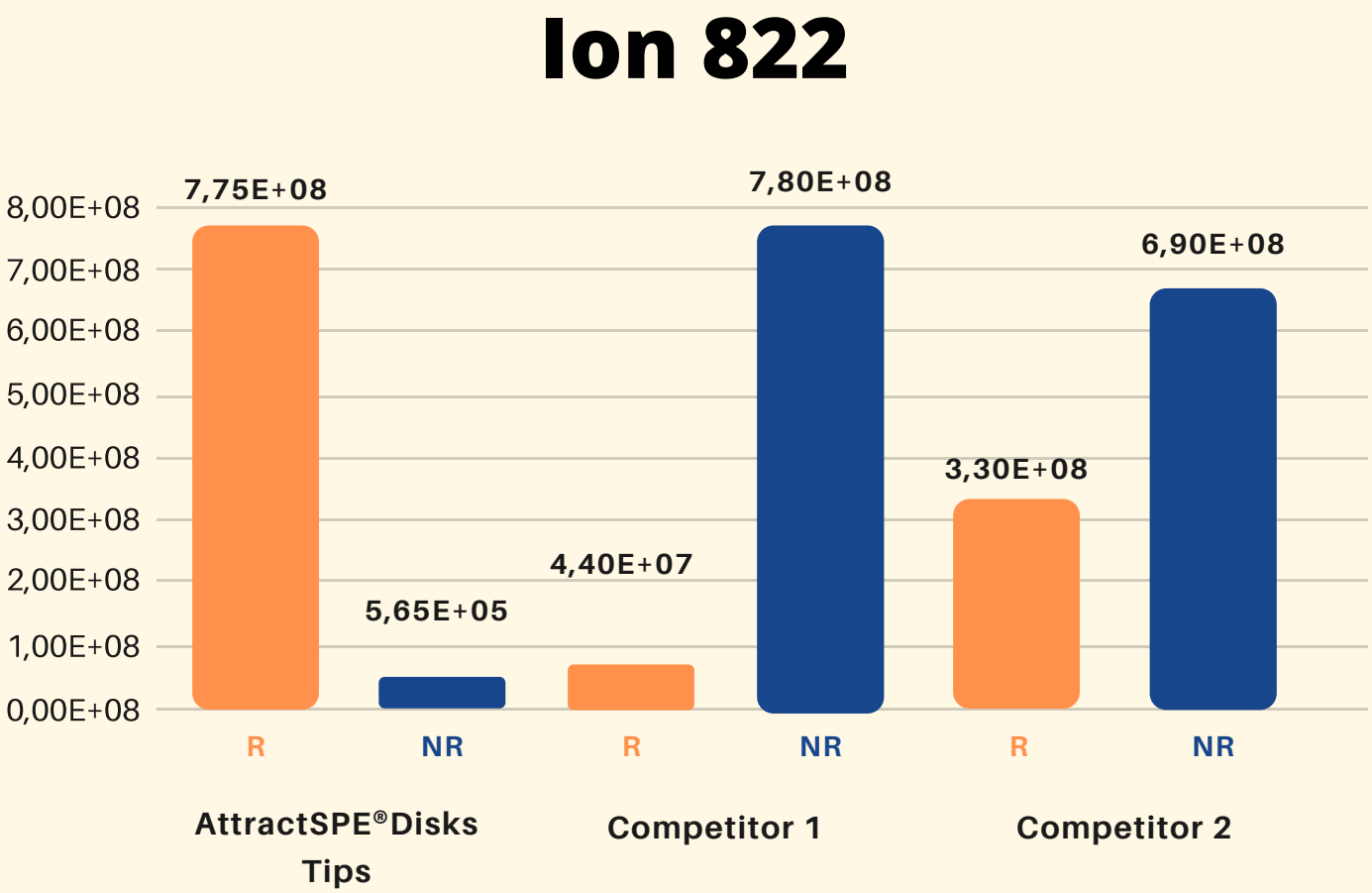
The intensity of two major ions of BSA, ions 722 and 822, were measured in each fraction, retained and not retained. This intensity gives information on the concentration of these peptides.

For ions 722 and 822, AttractSPE®Disks Tips shows a much higher intensity on the retained fraction in comparison to competitors products.

Indeed for ion 722, the intensity of retained fraction of AttractSPE®Disks Tips is 5 times the one of competitor 1 and twice the one of competitor 2. The same trend is observed for ion 822, the intensity of retained fraction of AttractSPE®Disks Tips is at least 10 times the one of competitor 1 and 2.3 times the one of competitor 2.

On the contrary, this data shows a very low intensity on the NOT-retained fraction AttractSPE®Disks Tips in comparison to each competitor. Indeed for ion 722, the intensity is at least 10 times lower than both competitors while for ion 822 this factor is at least 1000 times lower...
That means that AttractSPE®Disks Tips gives more information others.

Graph 2. Ions intensities for Ion 822

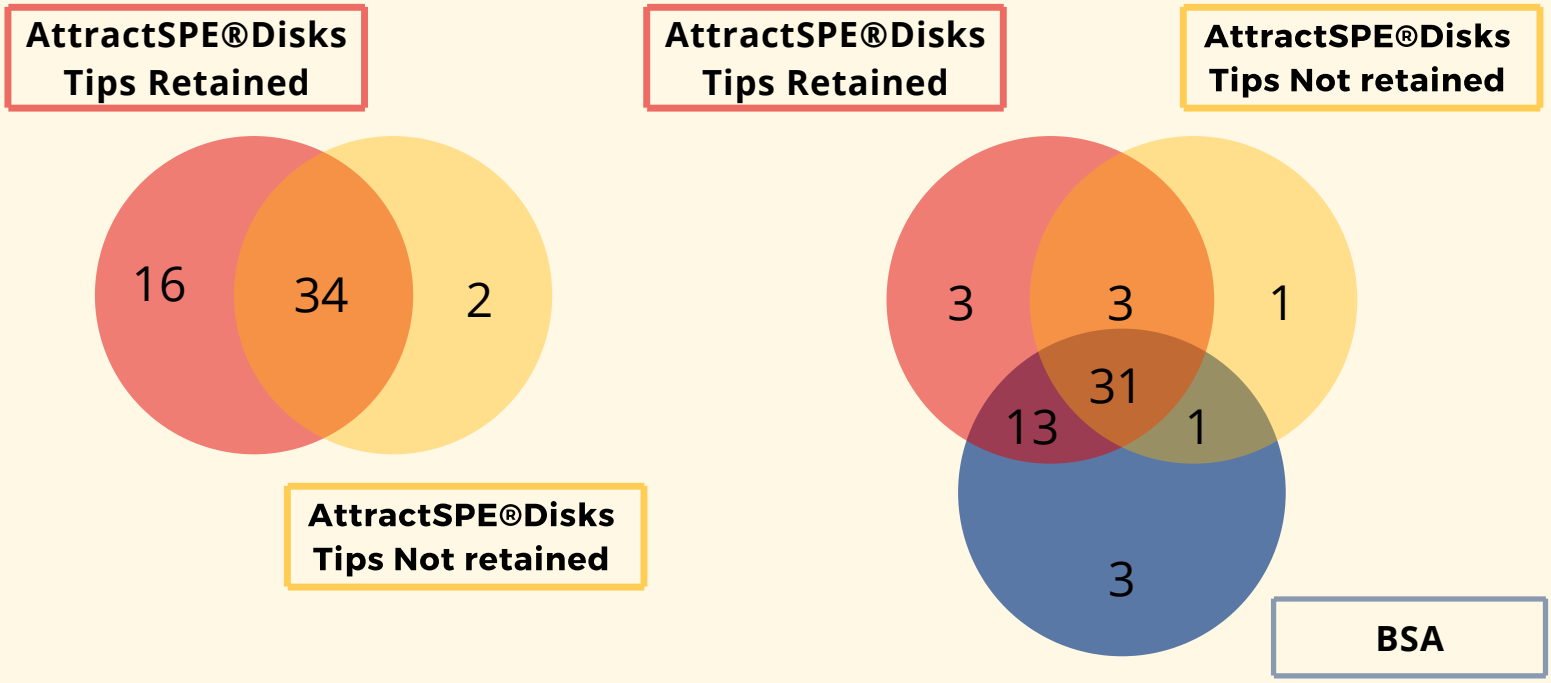


To conclude for AttractSPE®Disks Tips, the comparison of the intensity of two major BSA ions 722 and 822 shows a very high intensity of these ions for the retained fraction. Whereas the intensity is very low for the not retained fractions. This seems to indicate that even if some peptides could go through the columns during the loading, their concentration is very low.
SPE tips of competitors 1 and 2 retain much lower intensities of peptides.
Indeed, the concentration of Retained fraction is very high showing a good performance of AttractSPE®Disks tips. This also show that a peptide of low concentration in the loading solution could be more probably measured with AttractSPE®Disks Tips than with competitor products.

PEPTIDE ANALYSIS

Peptide analysis with Venn diagrams is made with peptides identified in at least two samples of the triplicate.

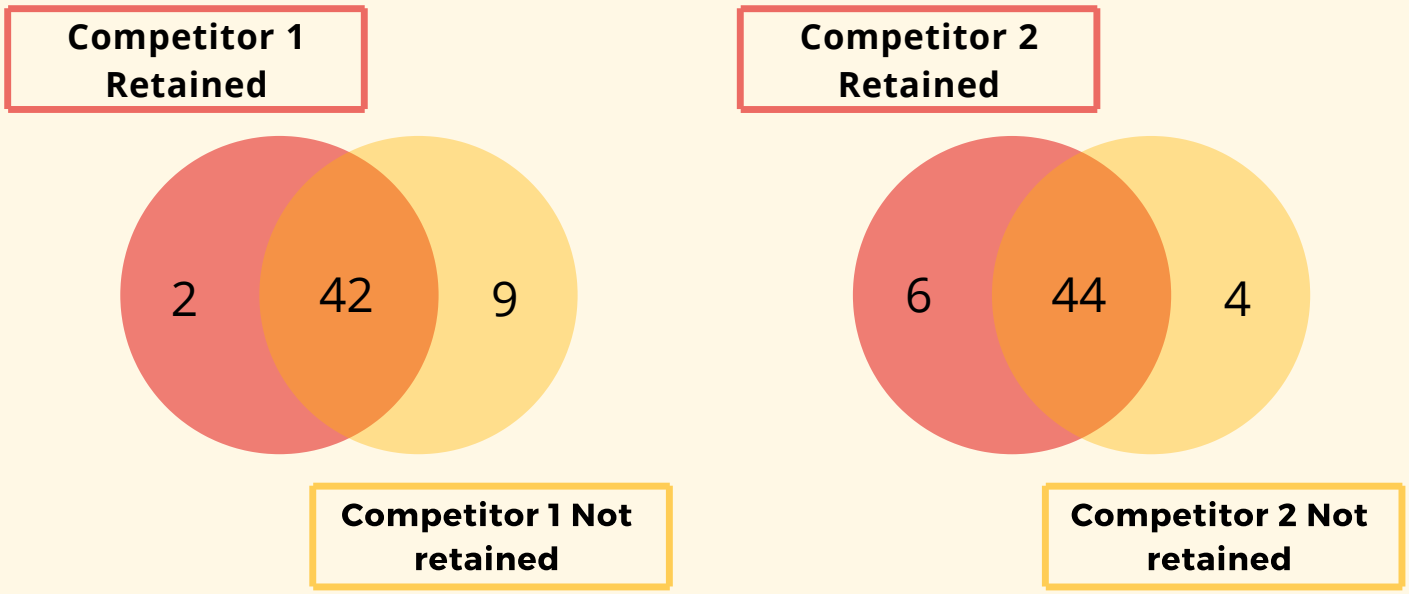
Graph 3. Venn diagrams for peptide analysis for AttractSPE®Disks Tips



For AttractSPE®Disks Tips, Venn diagrams (graph 3) show that 50 peptides have been identified in the retained fraction while 2 were exclusively not retained. This means that almost all peptides have been retained and can be used to identify the protein.

The comparison with BSA direct analysis show that 44 peptides of the retained fraction are common with BSA (over 48 BSA peptides identified). **So a large majority of peptides has been efficiently retained during the SPE.**

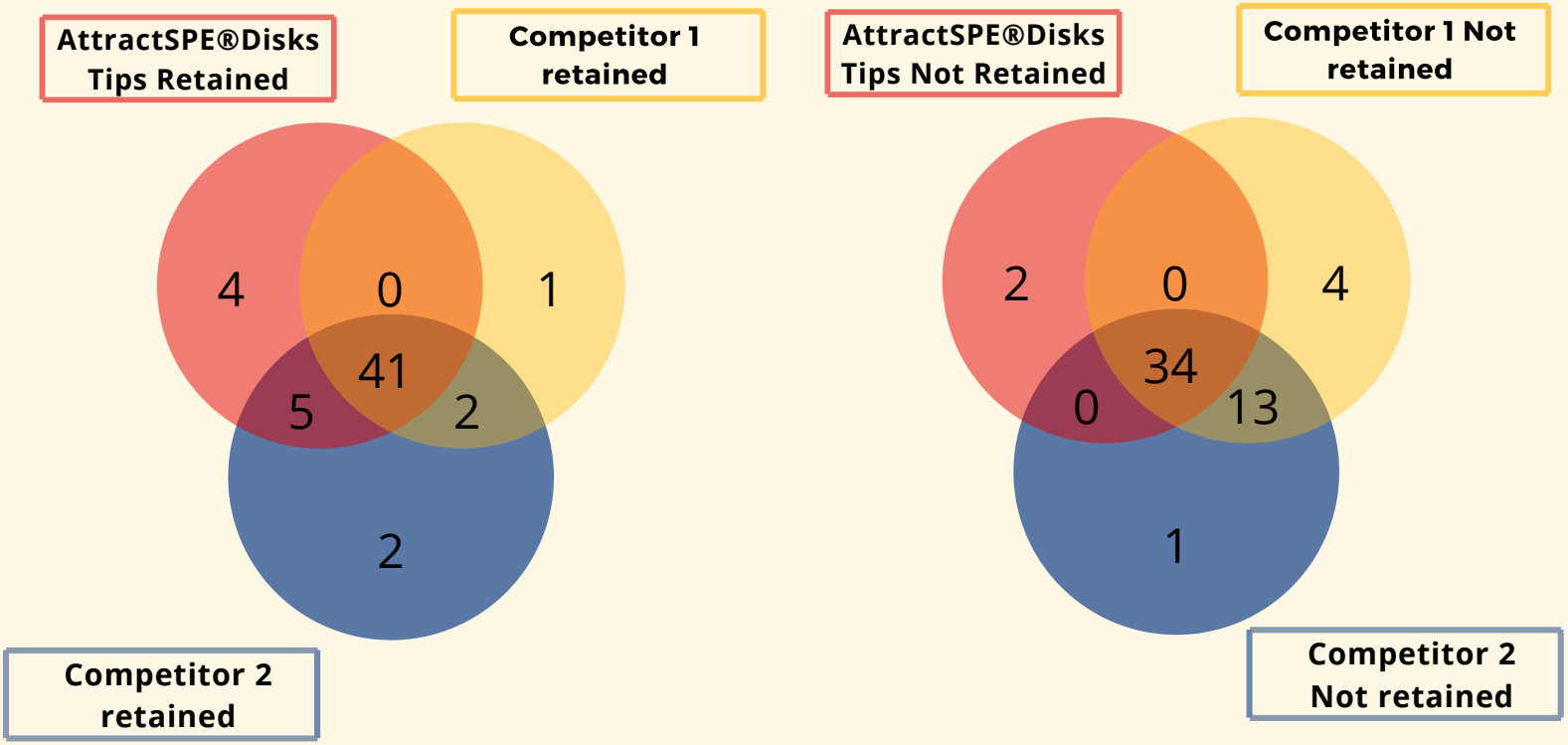
Graph 4. Venn diagrams for peptide analysis for competitors products



For competitor 1 SPE tips, Venn diagrams (graph 4) show that 44 peptides have been identified in the retained fraction while 9 were exclusively not retained.

For competitor 2 SPE tips, Venn diagrams (graph 4) show that 50 peptides have been identified in the retained fraction while 4 were exclusively not retained.

Graph 5. Venn diagrams for peptide analysis for competitors products



CONCLUSION

Performance of AttractSPE®Disks Tips

Thanks to all data generated by peptides analysis, **AttractSPE®Disks Tips shows excellent performance for peptidic purification.** Indeed, the percentage of coverage and PSM resulted to be high for the retained fraction. In addition, ion intensities of two majors BSA ions demonstrate a significantly higher concentration of the peptides in the retained fraction than in the not-retained fraction. Much less peptides and at low concentration are present in the not-retained fraction. In addition, most of these peptides are also present in the retained fraction. **So all important information is collected in the retained fraction.**

Comparison with both competitors

The comparison of AttractSPE®Disks Tips with two competitors products shows that competitor 1 is much less performing than other tested products. Lower PSM and %coverage as well as less peptides in Venn diagram.
The comparison of AttractSPE®Disks Tips with competitor 2 shows that **AttractSPE®Disks Tips is behaving better.** While %coverage and Venn diagram are quite similar, AttractSPE®Disks Tips have a 10% higher PSM and much higher Ion intensities on retained fraction, probably due to higher capacity of AttractSPE®Disks Tips, making possible the measure of low concentration peptides.

AttractSPE®Disks Tips C18 is efficient to retain and purify peptides and to make possible an easy and reliable identification of the proteins by LC/MS after digestion. Among tested SPE tips, AttractSPE®Disks Tips is the best performing for peptidic purification.

Part number of products used in this application note:		
Product :	**Quantity :**	**Part Number :**
AttractSPE® Disks Tips C18 - 200µL 96/pk	**96/pk**	**Tips-C18.T1.200.96**

Other formats of AttractSPE®Disks Tips using the same SPE disks (spin tube, 96 well plates, cartridges) and other sorbents (SDB-RPS, SDB-XC, HLB, C4, C8, silica...) make possible the fractionation, desalting of peptides or even proteins (due to various pore size).