OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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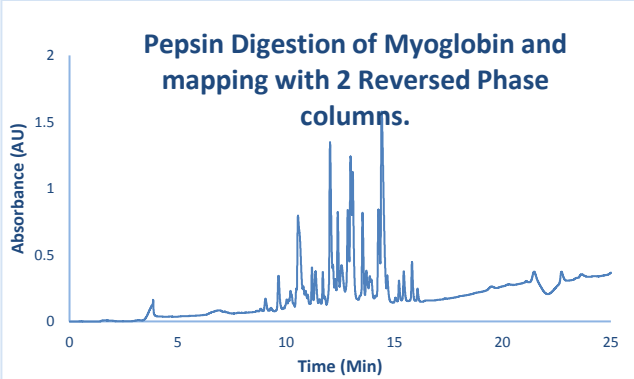
CAUTIONARY NOTE

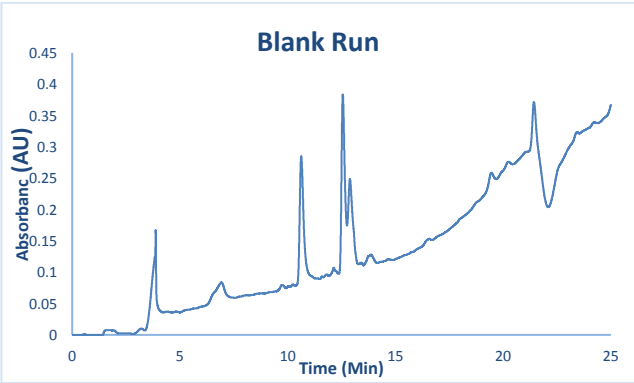
Background noise, not leaching enzyme.

It should be noted that the immobilized enzymes OraChrom provides are stables and do not leach.

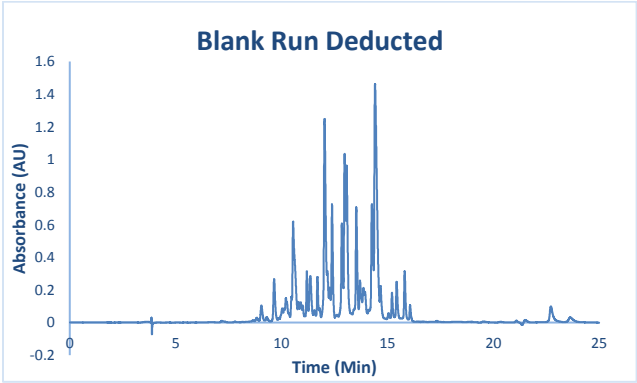
They can be used for the digestion of proteins for months and many runs.
We have considered the Pepsin digestion of Myoglobin from Application Note 160 and considered deducting the blank run associated with it.
The blank run consists of using the same set ups that are used during the digestion sequences and simply avoiding the injection of the protein to be digested.
In the present case of Myoglobin, the digestion is complete.
The digestion, trapping and ultimately mapping of the digested protein shows the following chromatogram.



However, if we were to run a blank, the resulting chromatogram reveals peaks that remain constant and can be considered as impurities from solvents, containers, salts, and instrument plumbing's.
At an absorbance of 210 nm they do not wash away or decrease as leached products with a number of injections.



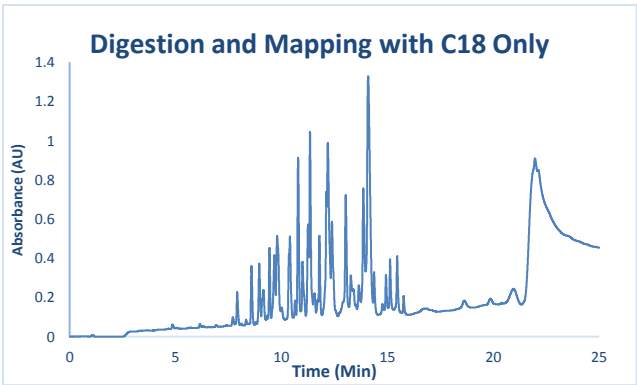
Should this blank run be deducted from the previous chromatogram, a chromatogram with flat baseline results that is consistent with full digestion of the protein.



To be noted that the enzyme column performs similarly after these runs and is not altered afterwards.

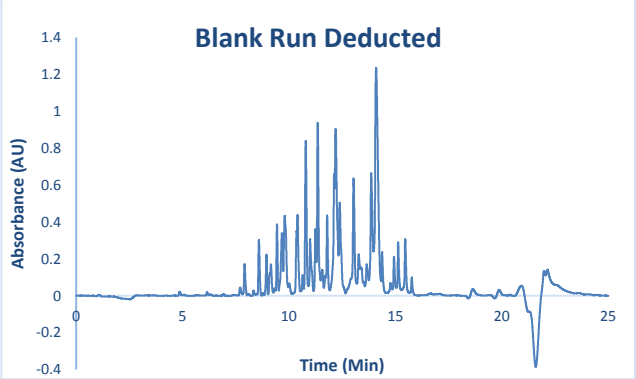
The concept of having a high capacity and pH stable polymeric added to the sequence provides the compatibility silica columns lack during digestions with high pH requirements enzymes such as Trypsin need.

In the following chromatograms we have avoided the use of the polymeric column.
The following chromatogram shows the retention of the resulting peptides and their mapping using the C18 silica column only.



If we were to run a blank as we did in the previous case, the resulting chromatogram will display similar absorbances.

Deducting it from the corresponding chromatogram, a similar profile emerges.



The protective nature of the polymeric column is therefore clear.
It protects the silica column without interfering with the mapping of the resulting peptide digests of the enzyme.

It is important to note that instrument manufacturers are aware of such phenomenon and provide ultra pure solvents, non leacheable containers and most anyting that reduces artifacts associated with the peaks that the chromatographer would notice.
A simple alternative would be to run a blank run and deduct it of the actual chromatogram.
The use of small bore columns would also help.
We have been using narrow bore columns of 2.1 mm ID to minimize the consumption of solvents while at the same time reducing the generation of waste.