

Technical Note

High Purity needed for reversed-phase separation of polypeptides

Reversed-phase high performance chromatography (RP-HPLC) is the most widely used chromatography technique for the separation and study of peptides. Peptides play a number of important roles in proteomics research and development in normal cell physiology and diseases. These short polymers of amino acids can act as the basic recognition unit for receptors and other biological binding proteins. Peptides are biologically important compounds that can serve e.g. as hormones, grow factors, neurotransmitters etc.

Peptides are also important structural and functional elements of proteins. For this reason the analysis of digests of proteins is a critical step in understanding the protein structure and is now an important quality control method in the biopharmaceutical laboratory.

The peptides are often present at very low concentrations and in a complex matrix of numerous other proteins, nucleic acids and small molecules. In order to get the full functional profile of the peptide it is important to characterize all amino acid side chains into four groups (basic, acidic, neutral, hydrophobic). The ionization properties can be dramatically influenced by the mobile phase composition such as organic phase modifier, ionic strength, pH, temperature and the capacity for ion-pair effects.

Trifluoroacetic Acid (TFA) as mobile phase modifier

By far the most commonly used ion-pair reagent for RP-HPLC of (poly)peptides and proteins is trifluoroacetic acid (TFA).

The "classical" gradient mobile phase system used in RP-HPLC of peptides and proteins consist of:

- 0.1%(v/v) TFA in water (mobile phase A)
- 0.1%(v/v) TFA in acetonitrile (mobile phase B)



TFA is a weak hydrophobic ion-pairing reagent that also serves to maintain a low pH. This low pH (~ 2) is necessary in order to protonate the peptide and to enhance the hydrophobicity of the peptide. The ion-pair reagent masks the positive charges of both the stationary phase and the peptide. Additional advantages of TFA include its high volatility (solvent removing is easy), and excellent optical properties.

Critical needs during the analysis:

TFA should be as pure as possible since even minor contaminants may be concentrated on the stationary phase and then eluted by the gradient.

A practical example is illustrated in Figure 1 on the next page. Two chromatograms are shown with 2 different qualities TFA (HPLC Grade and Analytical Grade). Fig 1. clearly demonstrated the importance of TFA purity.

For highly reproducible separations from run to run, fingerprinting for example, or from lab to lab, it is also essential to have a batch-to-batch consistency of the TFA purity and concentration in the mobile phase.

Analytical Conditions

Column	BAKERBOND™ Wide Pore C ₁₈ , 5µm, 250 mm L x 4.6 mm I.D. (PN 7104-00)
Mobile Phase	0.1%(v/v) TFA (PN 9470) in H ₂ O (PN 4218) (A); 0.1%(v/v) TFA (PN 9470) in Acetonitrile (PN 9017) (B)
Flow Rate	1 ml/min
Gradient	5% A (10 min), to 60% A in 30 min (linear gradient), 60% A (10 min)
Temperature	25°C
Detection	UV at 215 nm

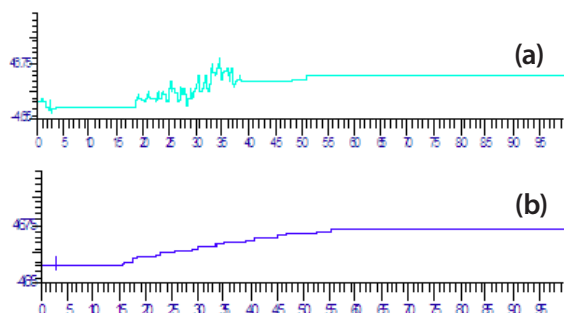


Figure 1 Effect of TFA purity on baseline absorbance with 0.1% (v/v) TFA
 a. 0.1% TFA analytical grade
 b. 0.1% TFA J.T. Baker HPLC Analyzed grade (PN 9470)

The J.T. BAKER® solution for these critical needs

J.T. Baker uses very high quality TFA with an assay of min. 99.5% Certified processes during production guarantee high purity and a lot-to-lot consistency.

Also every batch of J.T. BAKER HPLC Grade TFA (9470) is tested and specified for protein/peptide gradient elution. The result of the gradient elution profile is actually printed in the certificate of analysis.

1 ml Ampoules for TFA, easy, accurate, time and storage saving:

J.T. BAKER HPLC Grade TFA (9470) is also available in a 1 ml ampoule. Simply dilute with one liter HPLC water (4218) or acetonitrile (9017) and you have a ready-to-use 0.1% (v/v) TFA solution.

These handy ampoules are packed with 2 or 10 into a box containing also a certificate of analyses and material for opening the ampoule. And of course, a box containing 10 x 1 ml ampoules saves a lot of storage space compared to 10 L solution.

Product Number	Description	Pack Sizes
9470	Trifluoroacetic Acid, HPLC	1 x 70 ml Ampoule; 10 x 1 ml Ampoule
9810	Trifluoroacetic Acid, LCMS	10 x 1 ml Ampoule; 2 x 1 ml Ampoule
9820	Formic Acid, LCMS	10 x 1 ml Ampoule; 2 x 1 ml Ampoule



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Avantor products are used in a wide range of industries. Our biomedical and life science solutions are used in academic, industry and quality control laboratories for research, pharmaceutical production and medical lab testing, while our electronics solutions are used in the manufacturing of semiconductors, photovoltaic cells and flat panel displays. Based in Center Valley, Pennsylvania (USA), Avantor is owned by an affiliate of New Mountain Capital, LLC.

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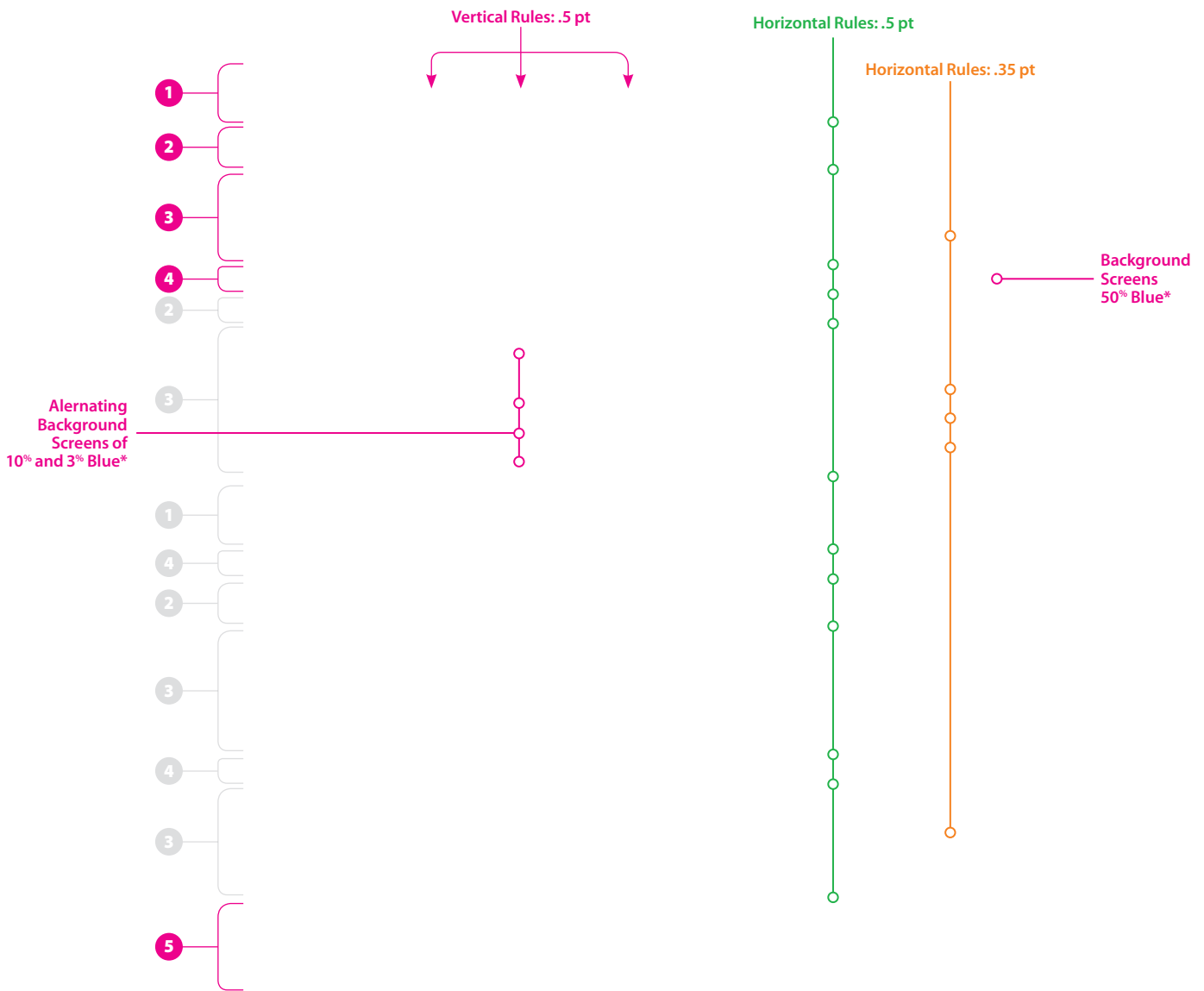
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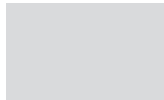


Technical *Note*



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1	Myriad Pro Semibold	10	11	-10	100% Blue*	Bottom	0p8	0p5
2	Myriad Pro Semibold	7.5	8	-10	100% Black	Bottom	0p3	0p4
3	Myriad Pro Regular	7.5	8	-15	100% Black	Top	0p3	0p4
4	Myriad Pro Semibold	8	9	-10	100% White	Top	0p3	0p4
5	Myriad Pro Regular	5.5	6	-10	100% Black	Top	0p4	1p0

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*Avantor™ Blue: PMS: 7461 C CMYK: 95/37/05/0 RGB: 0/130/192 HEX: 0099cc	Avantor Green: PMS: 376 C CMYK: 60/04/100/0 RGB: 116/184/67 HEX: 74b843	Solid Black PMS: Process Black CMYK: 0/0/0/100 RGB: 0/0/0 HEX: 000000	40% Black PMS: Cool Gray 6 CMYK: 0/0/0/40 RGB: 170/170/170 HEX: aaaaaa

