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Waters Chromatography Chemistry

Waters Delta-Pak™ High Pressure Inert (HPI) Column.

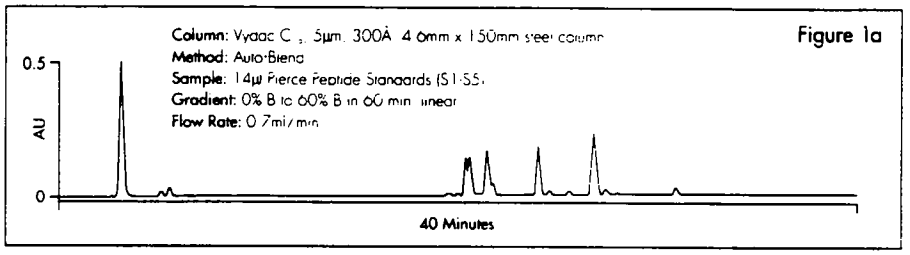
Unequaled quality and consistency of resolution.

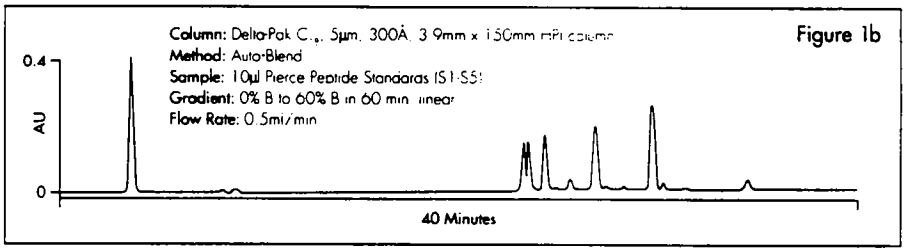
Waters Delta-Pak HPI Column is ideal for the complex separations required in high resolution analyses and is ideally suited for purification of peptides and proteins. Delta-Pak HPI is based on a highly stable, C_{18}-bonded and fully end-capped 5µm spherical silica, with 300Å pore size. The separations of complex peptide mixtures derived by enzymatic or chemical cleavage of proteins achieved with the Delta-Pak HPI column rivals and often exceeds the performance of a similar C_{18} chemistry from Vydac™. This is demonstrated by the superior resolution of peptide retention standards (Figure 1a & b).

Extend your mobile phase options with a metal free column.

Delta-Pak C_{18} chemistry packed in a high performance, non-metallic column extends the mobile phase options to modifiers generally avoided in standard chromatography systems. In this advanced column design, the sample contacts polyetheretherketone (PEEK) and ultrahigh molecular weight polyethylene, two materials that are widely accepted for their biocompatibility and inertness. Since PEEK columns are capable of high pressure operation, they can be used for the same applications with the same chromatographic performance as steel columns–with the added advantage of chemical inertness.

Figure 1a & b: Comparison of Waters Delta-Pak C_{18} and Vydac C_{18} chemistries for resolution of peptide standards.





Waters high performance Delta-Pak chemistry in a non-metallic column configuration provides enhanced resolution of closely-related peptide compounds. The sample, Peptide Retention Standards, contains five decapeptide sequence variations in positions 3 & 4. In addition, one has a free amino group. Both separations were performed at the same linear velocity and gradients were generated with the same number of column volumes.

Waters Auto·Blend™ Method:

- Automatically blends up to four solvents/buffers.
- Automatically switches between TFA and HCl-based mobile phases.
- For all the chromatography shown here, the following Auto·Blend method was used:

  A: 0.1% TFA in H_2O
  B: 0.1% TFA in CH_3CN
  C: 0.02% HCl in H_2O
  D: 0.02% HCl in CH_3CN

*All chromatography was performed on a Waters 625 LC system and Waters 994 Photodiode Array Detector at 214nm except where indicated.

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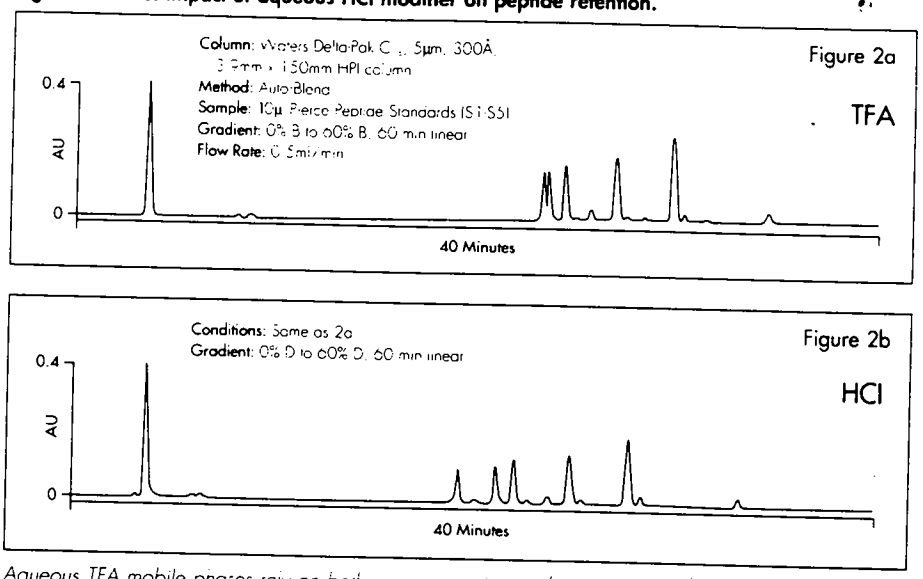
Select novel mobile phase modifiers to improve separations.

Peptide mapping is commonly performed using gradient reversed phase chromatography in the presence of aqueous trifluoroacetic acid (TFA). Alternative packings can provide different selectivities, but it is more practical to modify the separation by changing the mobile phase conditions. Furthermore, recent demands for higher sensitivity detection and an increasing focus on spectral data acquisition have stimulated evaluation of alternative aqueous modifiers.

Dilute hydrochloric acid (HCl) is an alternative to TFA since it has better optical clarity in the low UV and provides different separation selectivity for peptide separations. The chromatograms in Figure 2a & b illustrate the altered selectivity achievable through the use of HCl as a modifier.

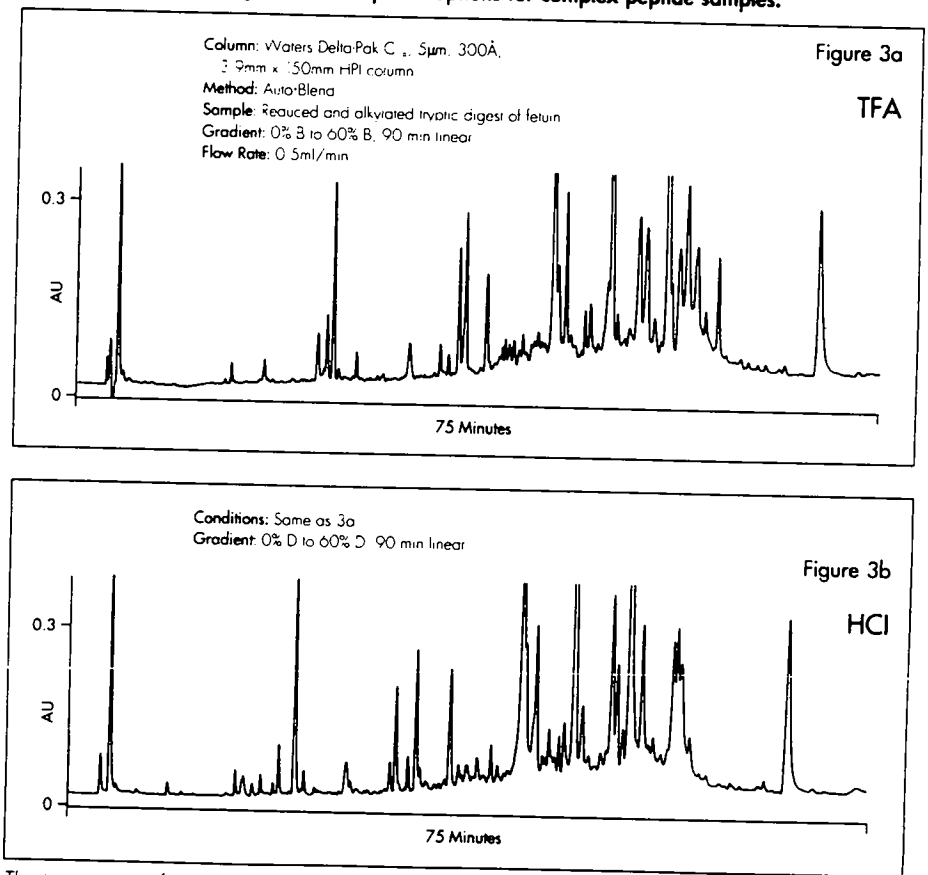
Figures 2a & b: Impact of aqueous HCl modifier on peptide retention.



Aqueous TFA mobile phases rely on both ion suppression and ion pairing to facilitate peptide retention on reversed phase columns. Use of aqueous HCl as a mobile phase modifier changes separation selectivity by maintaining ion suppression in the absence of hydrophobic ion pairing.

Another example of altered selectivity due to the aqueous modifier is apparent by comparing Figure 3a (TFA) & b (HCl), a tryptic digest of fetuin. When used in parallel experiments, these reagents offer complementary selectivities to provide more information about complex peptide mixtures.

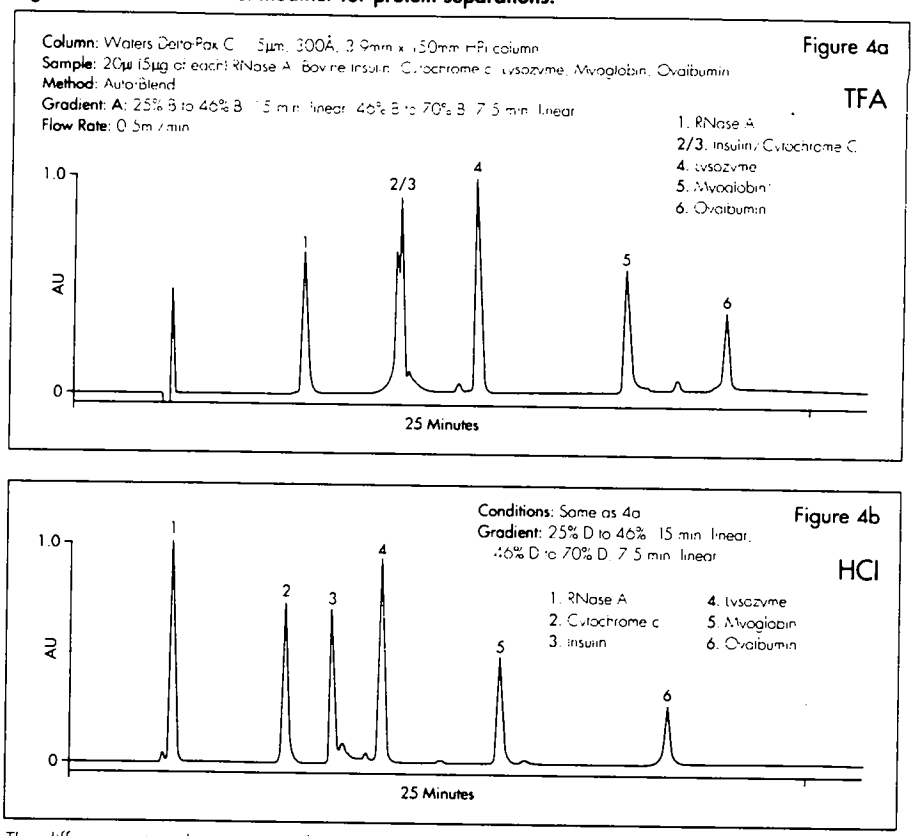
Figure 3a & b: Advantages of mobile phase options for complex peptide samples.



The importance of mobile phase options is apparent with complex peptide maps. Whether the goal of the separation is to prepare pure peptides for sequencing or to fingerprint proteins, it is imperative that alternative conditions be evaluated to assure complete resolution of all peptides.

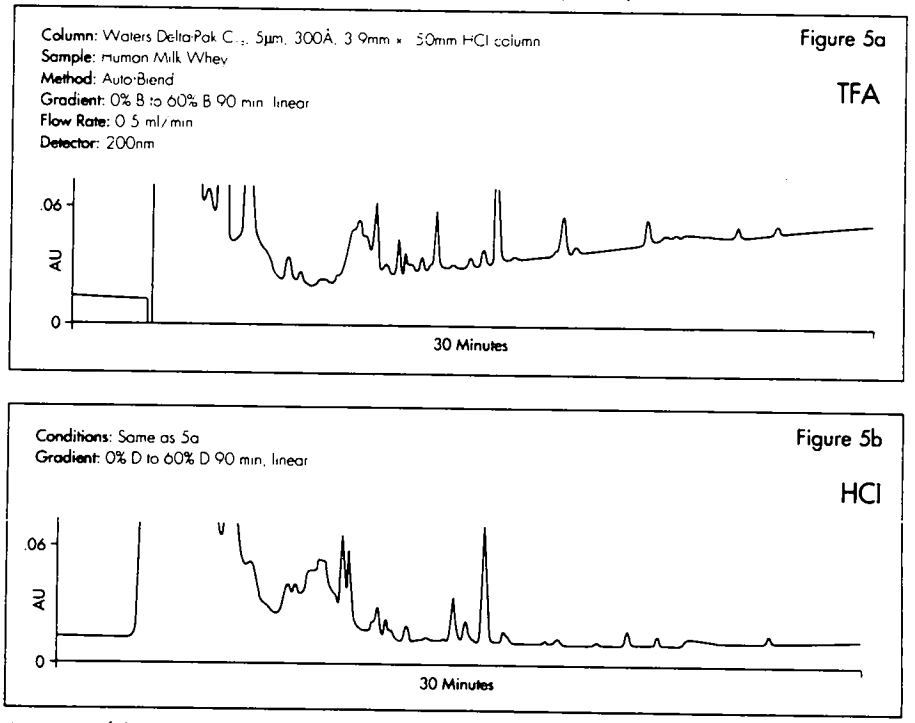
The advantages of alternative mobile phase modifiers are also applicable to the separations of larger macromolecules such as proteins (Figures 4a & b, 5a & b). Reversed phase separations are based largely on the hydrophobic nature of the protein or peptide. Thus, reversed phase separations are frequently used as additional steps in protein purification. Following reversed phase separation, proteins are collected in volatile eluents that are compatible with subsequent analytical procedures such as amino acid analysis or sequencing. Larger quantities of proteins or peptides may also be isolated using scalable Delta-Pak chemistry and HCl as a modifier.

Figure 4a & b: Use of HCl modifier for protein separations.



The differences in selectivity can also be demonstrated when separating protein standards. Figure 4a shows a protein standard mixture using TFA. Bovine insulin and Cytochrome c are poorly resolved using the TFA modifier. The different selectivity using HCl as a modifier results in increased resolution of this pair of proteins. Also note the shift to earlier retention times with HCl.

Figure 5a & b: Impact of aqueous modifier changes on complex separations.



A portion of the separation of human milk whey demonstrates the use of aqueous HCl to provide alternative selectivity and optical clarity as apparent in the reduced baseline shift over the gradient when monitored at 200 nm.

Purify your peptides with a scalable chemistry using HCl.

Waters offering of scalable chemistries include PrepPak® polyethylene cartridge columns that are radially compressed by Waters RCM™ 25x10 cartridge holder to provide longer lifetime and improved bed stability at an economical price. The cartridge columns are available packed with 15µm Delta-Pak in 25mm i.d. x 100mm lengths. Connect one, two or three 25mm x 100mm PrepPak cartridges together to get the optimum column length, volume and efficiency for your purification.

Advanced chemistries complement Waters 625 LC System.

Waters 625 LC System combines advanced polymeric technology and low dispersion system volume into a single liquid chromatograph, providing the highest performance available in a non-metallic system. This instrument is designed to handle a wide variety of life science applications including high resolution protein purification and microbore peptide mapping.

Waters exclusive Auto·Blend method, used in conjunction with the 625 LC System, increases laboratory productivity through rapid methods optimization and eluent switching. The combination of advanced system and column design facilitate the development of better separations by providing convenient, economical and rapid ways to alter chromatographic selectivity.

Ordering Information.

Item	Part No.
Waters Delta-Pak C_{18}, 5µm, 300Å 3.9mm x 150mm HPI Column	PN35571
Waters PrepPak® 25x10 cartridges Delta-Pak C_{18}, 15µm, 300Å	PN38507
Waters PrepPak 25x10 Cartridge Holder (non-metallic)	PN15814
Prep Guard-Pak™ Inserts (2/pkg) Delta-Pak C_{18}, 300Å	PN38522
Waters 625 LC System includes Waters 625 Fluid Handling Unit, 625E Powerline™ Controller, system rack and variable volume injector.	PN88701

Adapt your LC system for peptide mapping with HCl.

To adapt your existing LC system to use with HCl, Waters offers the HPI Conversion Kit, an assembly of tubing, fittings and ferrules for use in modifying your stainless steel HPLC system fluid path.

Item	Part No.
HPI Conversion Kit	PN35134

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