Essentials in biore

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A New Methodology for Peptide Mapping

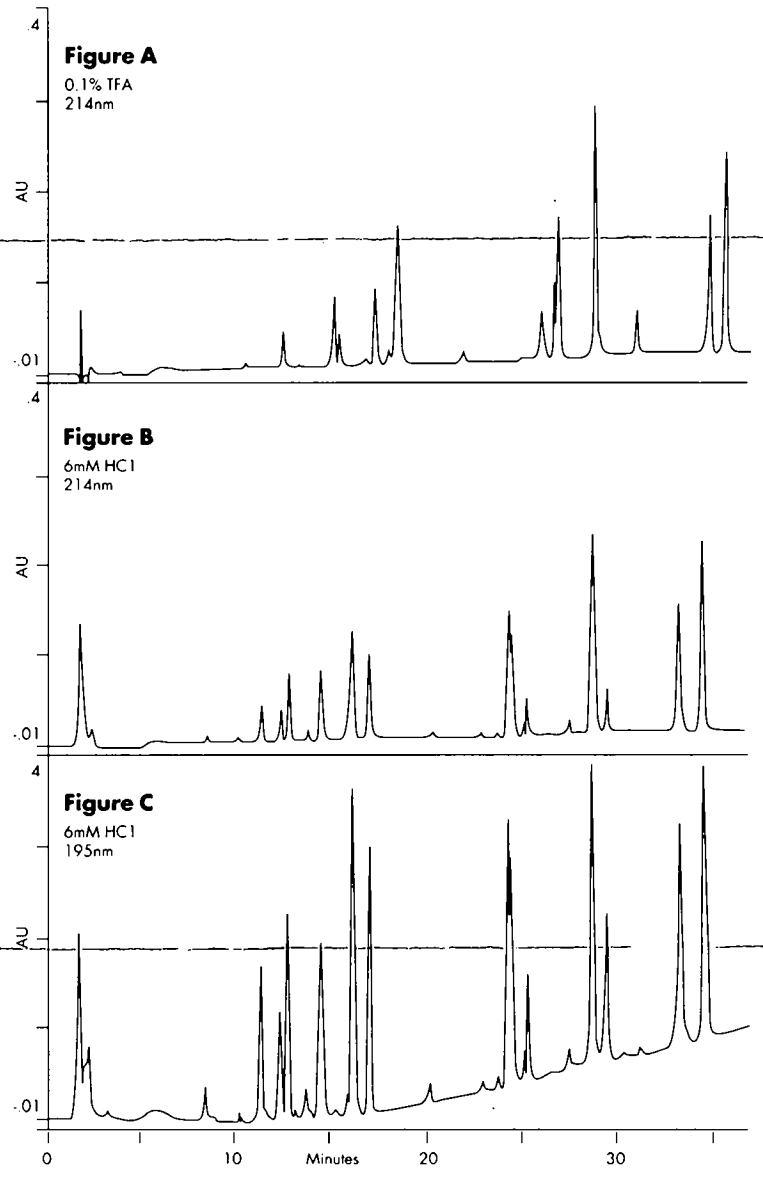
Exciting alternative to TFA

Peptide mapping is commonly performed using gradient reversed phase chromatography in the presence of trifluoroacetic acid (TFA). The mixtures of peptides obtained in digests of large proteins are often too complex to ensure resolution of each peptide. Alternate packings can provide different selectivities, but it is more practical to optimize 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. The introduction of a high performance non-metallic column packed with Waters Delta-Pak™ 300Å C_{18} extends the mobile phase options to modifiers generally avoided in standard chromatography systems. Dilute hydrochloric acid (HCl) is an alternative to TFA that is volatile, has better optical clarity in the low UV, and provides different separation selectivity for peptide mapping. To test the utility of HCl, a tryptic digest of chicken Cytochrome c was separated in the presence of TFA and HCl. The high resolution maps were achieved using Waters new non-metallic column run on a Waters 625 LC system.

HCl provides high resolution maps

The altered selectivity due to the aqueous modifier is apparent by comparison of figures A (TFA) and B (HCl). Most of the peptides are more strongly retained in the presence of TFA than in HCl. In a pH 2 mobile phase containing TFA (or other fluorinated organic acid), peptide carboxyl groups

Tryptic Digest of Cytochrome *c*



Chicken Cytochrome c tryptic digest is chromatographed using conventional 0.1% TFA as the mobile phase modifier in figure A. The selectivity in using HCl as an alternative to TFA is demonstrated in figure B. The enhanced optical clarity of HCl is evident in Figure C, monitored at 195nm (conditions on back).

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are protonated and TFA ion pairs are formed. Dilute HCl is an alternative modifier that maintains ion suppression in the absence of ion pairing. Both reagents yield useful peptide maps with numerous differences in selectivity. Significantly, the large peak (18 min. in TFA, known to include two peptides) is resolved into two peaks (16 and 17 min.) with HCl. When used in parallel experiments, these reagents will offer complementary selectivities to provide more information about complex peptide mixtures.

Dramatic enhancement of sensitivity

At 214nm, the optical clarity of HCl (figure B) when compared to TFA (figure A) is apparent in the reduced baseline shift over the gradient. Furthermore, detection is more sensitive at lower wavelengths where side chains of aliphatic amino acids contribute to the spectrum. With HCl, peak heights are increased by as much as 4 fold at 195nm (figure C) when compared to 214nm (figure B). The optical clarity of mobile phases based on HCl extends the usable wavelength range thus providing enhanced sensitivity in the low UV for peptide detection and characterization.

Better separations, enhanced sensitivity, expanded spectral data

The use of HCl as mobile phase modifier for peptide mapping extends the repertoire of the protein chemist in three ways. It facilitates the development of better separations by providing a convenient, economical and quick way to alter chromatographic selectivity. It provides enhanced sensitivity because its minimal UV absorbance is compatible with monitoring at low wavelengths. Finally, it facilitates analysis of the unique spectral properties of peptides by extending the usable range to the low wavelengths where many amino acid side chains absorb.

System and column are optimized for HCl applications

The Waters 625 LC System combines advanced polymeric technology and low dispersion system volume into a single liquid chromatograph optimized for high performance peptide mapping. Waters new non-metallic Delta-Pak™ column complements the 625 LC to allow the selection of novel mobile phase modifiers to improve chromatographic performance.

Ordering Information

Waters 625 LC System includes Waters 625 Fluid Handling Unit, 625E PowerLine™ Controller, system rack, and variable volume injector	P/N 88701
Waters 991 Photodiode Array Detector with PowerMate® 386SX Plus Computer	P/N 34016
991 PowerLine control	P/N 31381
991 non-metallic flow cell	P/N 34011
Waters Delta-Pak C_{18} Column, 5µ, 300Å 3.9mm x 150mm, non-metallic	P/N 35571

Conditions (from front) Cytochrome c (1mg/ml 0.4M Ammonium Bicarbonate, pH 8) was digested with trypsin (10:1 (w/w), respectively) for 24h at 37° C. Samples were diluted 1:10 with aqueous TFA or HCl prior to separation on a Waters 625 LC System monitored at 190-425nm with a Waters 991 Photodiode Array Detector. The digest was separated on a non-metallic column (3.9mm x 150mm) packed with Waters Delta-Pak™ C_{18}, 300Å, 5µ, at 1ml/min. at 35° C. The separation gradient was 0-60% acetonitrile over 33 column volumes (60 minutes) with a constant concentration of mobile phase modifier, either 0.1% TFA or 6mM HCl.

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