OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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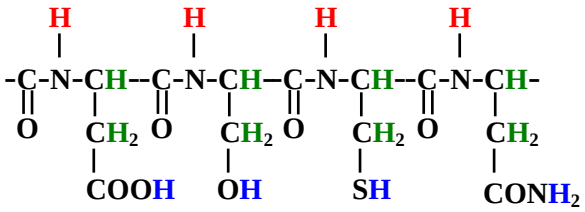
APPLICATION NOTE

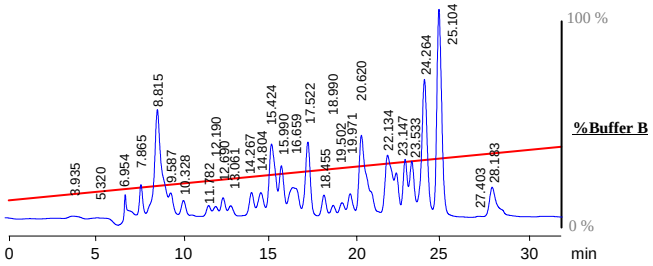
StyrosZyme™ Pepsin, Immobilized Enzyme on Polymeric Hard Gel Stationary Phase: On line digestion at 0º C and pH 2.5.

The study of proteins with Hydrogen Exchange and Mass Spectroscopy is an important method in deciphering their structure and acquiring information about their dynamics.
This technique has been refined since its early start in 1993. It is now applied to a large number of proteins as markers of different diseases. It also provides a very unique way to acquire crucial information about the structure of these proteins.
Considering the protein structure, one can identify three types of hydrogen based on the exchange rates with its heavier isotope deuterium. The backbone amide hydrogens identified in red color, provide with a measurable rate exchange and therefore can be used for this specific purpose. The other types of hydrogens, identified as blue and green, exchange at either too fast a rate or exchange at extremely slow rates.

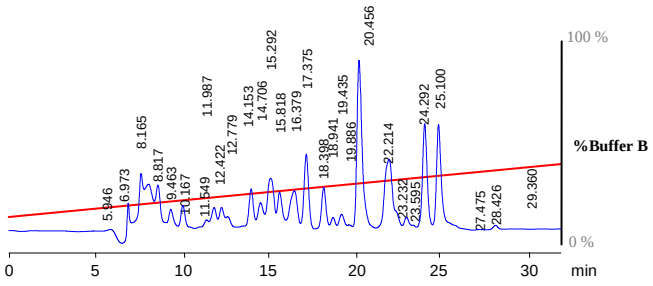


The exchange rates of the semi-labile hydrogens of the amide backbone are subject to solvent accessibility which itself depends on protein structure. By measuring the added weight after controlled exchange with deuterium using high resolution mass spectrometers, it is possible to derive clear concepts about the dynamics of proteins. Furthermore since the amide hydrogens are involved in hydrogen bond formation of alpha helices and beta sheets of the secondary structure of proteins, their exchange rates is a valuable means in detecting those structures.
The proteins can also be analyzed as peptides fragments after deuterium exchange. StyrosZyme™ Pepsin columns provide the possibility of online digestion in a short period of time without the interference of any leftover enzyme in the sample. StyrosZyme™ Pepsin is an immobilized enzyme on polymeric that can operate at 0º C and low pHs of 2.5. Under these conditions the average half-life of back exchange for the average amide deuterium is between 30 to 60 minutes allowing plenty of time for an online digestion.

The following chromatograms show the online digestion of a sample of Cytochrome c from horse heart at 0 and 20º C.



Chromatogram 1
Peptide digests from Cytochrome c from horse heart at 0º C separated on a STYROS™ 2R/XH 4.6 X 150 mm at 0.5 ml/min (180 cm/hr)



Chromatogram 2
Peptide digests from Cytochrome c from horse heart at 20º C separated on a STYROS™ 2R/XH 4.6 X 150 mm at 0.5 ml/min (180 cm/hr)

In both cases the digestion is over 97% complete. The difference lies in the ratios of the peptide fragments.
The online digestion in the StyrosZyme™ Pepsin column is run at a linear flow rate of 9 cm/hr during a 10 minute run. The column is stable at this pH and temperature.

Table 1. Operating parameters for the chromatograms.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	StyrosZyme™ Pepsin 2.1 x 50 mm STYROS™ 2R/XH 4.6 X 150 mm
Mobile phase for reversed phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN:H2O (95:5)
Mobile phase for digestion.	100 mM Phosphate + 150 mM NaCl in DI H2O at pH=2..5
Flow rate	As indicated.
Gradient	As indicated
Temperature	0°C and 20º C for digestion. 20º C for separation.
Detection	214 nm
Injection volume	2 µl
Sample:	10 mg/ml Cytochrome c in mobile phase buffer A.