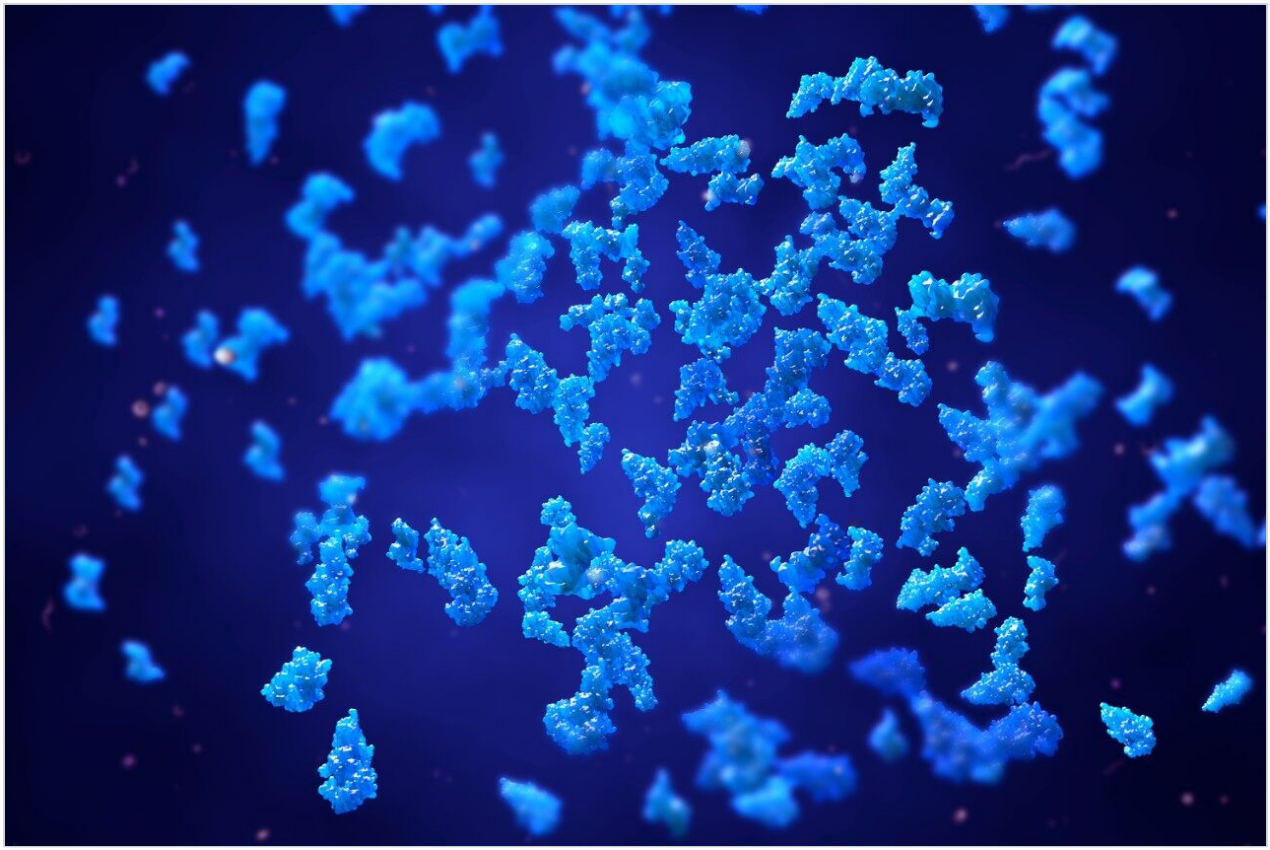
Waters™

응용 자료

*Rapi*Gest SF Accelerated Tryptic Digestion of Proteins

Waters Corporation



Abstract

This application note demonstrates *Rapi*Gest SF accelerated tryptic digestion of proteins.

Introduction

*Rapi*Gest SF enhances solubility and enzymatic digestion of proteins

Surfactants are often applied for solubilization of biopolymers such as proteins. *Rapi*Gest SF is a surfactant designed to enhance in-solution enzymatic digestion of proteins. It solubilizes proteins and makes them more susceptible to enzymatic proteolysis without inhibiting enzyme activity. Unlike the other common surfactants, *Rapi*Gest SF is compatible with various enzymes such as trypsin, chymotrypsin, Lys-C and Glu-C. This surfactant can be easily removed after digestion via acid degradation (Fig 1), eliminating laborious sample preparation prior to the MALDI-Tof MS, HPLC-MS or HPLC/UV analysis.

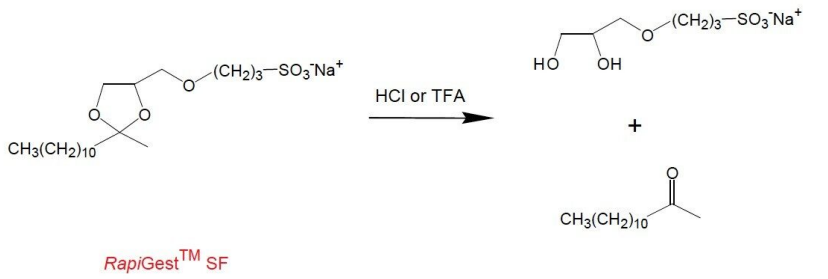


Figure 1. RapiGest SF removal from solution.

Experimental

*Rapi*Gest SF is compatible with mass spectrometry and LC-MS analysis

In general, it is imperative to remove surfactants (such as SDS) prior to liquid chromatography or mass spectrometry analysis. *Rapi*Gest SF can be removed by treating it with acids. Surfactant rapidly decomposes into two products, sodium-3-(2,3-dihydroxypropoxy) propanesulfonate and dodeca-2-one at acidic conditions. The $t_{1/2}$ at pH 2 is about 8 minutes. Dodeca-2-one is water immiscible, hence, it can be removed by centrifugation. Aqueous fraction can be directly analyzed by HPLC, LC-MS or by MALDI-Tof MS. At low *Rapi*Gest SF concentrations (<0.05%) the *Rapi*Gest SF degradation prior to MALDI-MS or LC-MS analysis is optional.

*Rapi*Gest SF degradation procedure after the protein digestion

1. Add strong acid to the sample (final conc. 30–50mM HCl, pH ~2). Alternatively add trifluoroacetic acid (final conc. 0.5%, pH ~2).
2. Incubate the sample at 37 °C for 30 minutes.
3. Centrifuge the sample to remove the water immiscible degradation product (Figure 1) at 13,000 rpm for 10 minutes. Analyze the aqueous fraction by LC-MS or MALDI-MS.

Example of tryptic digestion conditions for globular proteins

Protein concentration:	Horse Myoglobin, 2.0 µg/µL in 50 mM NH_4HCO_3
Myoglobin:Trypsin ratio:	100:1 (w:w)
Incubation temperature:	37 °C
Reaction time:	15–60 minutes

Results and Discussion

Example of Rapid digestion of globular proteins (Horse Myoglobin)

*Rapi*Gest SF helps to achieve a rapid and complete in-solution enzymatic digestion of proteolytically rather resistant proteins such as horse myoglobin (Figure 2). Using traditional protocol, majority of the protein remains undigested even after long hours of incubation with trypsin (Figure 2B). Using *Rapi*Gest SF, a complete tryptic digestion of horse myoglobin was achieved in minutes instead of hours (Figure 2A). Myoglobin was solubilized in 0.1% of *Rapi*Gest SF in 50 mM ammonium bicarbonate buffer, pH 7.9 and digested with trypsin (Promega) at 37 °C. The protein to trypsin ratio was 50:1 (w:w).

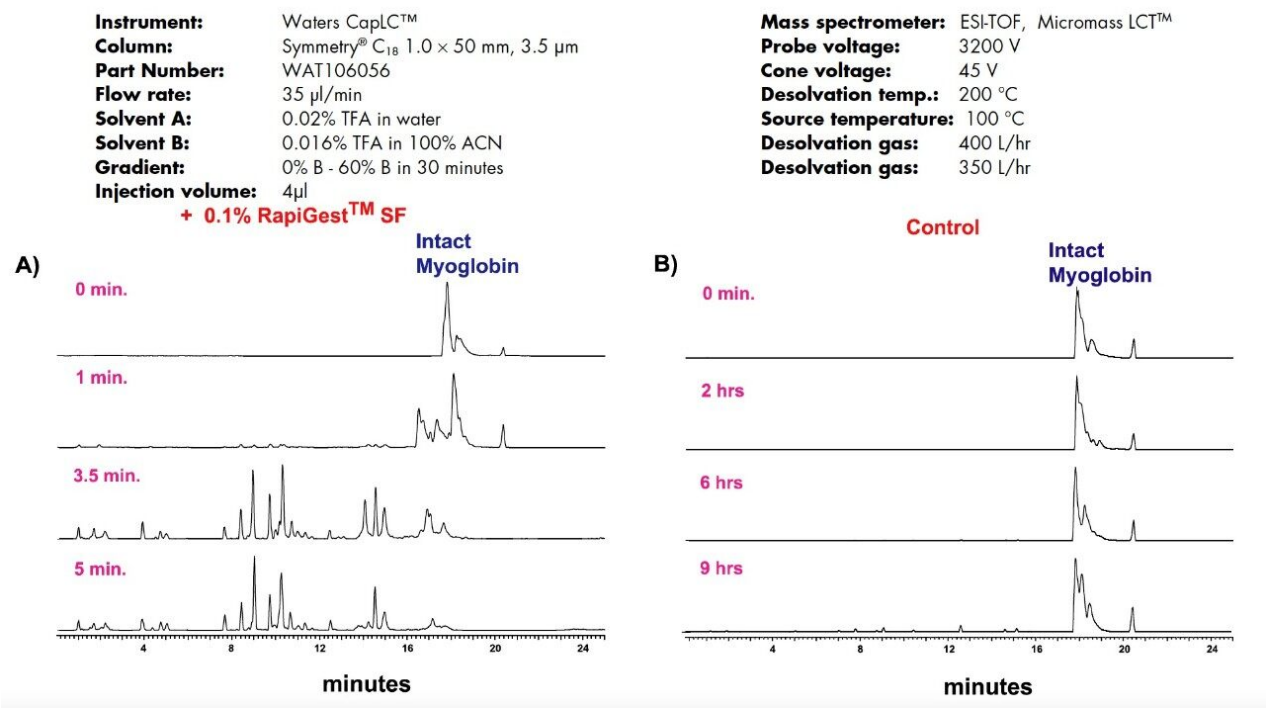


Figure 2. LC-MS chromatograms of tryptic digested horse myoglobin. (A) Horse myoglobin solubilized in 0.1% RapiGest SF (w/v) was completely digested within 5 minutes. (B) Without the use of 0.1% RapiGest SF, a majority of myoglobin remained undigested after 9 hrs.

How *Rapi*Gest SF speeds up the in-solution Protein Enzymatic Digestion

Many hydrophobic proteins are resistant to proteolysis because their cleavage sites are inaccessible to endoproteases. *Rapi*Gest SF is a trypsin-compatible surfactant that solubilizes and unfolds the protein substrates making them more amenable to cleavage. *Rapi*Gest SF can be incorporated to the traditional protein enzymatic digestion protocol by simply adding the *Rapi*Gest SF to the sample buffer. The recommended concentrations of *Rapi*Gest SF are between 0.025% to 0.1% (w/v) depending on the hydrophobicity of the substrates.

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RapiGest SF Surfactant <https://www.waters.com/waters/1000941>

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