

APPLICATIONS

Comparison of Chaotropic Reagents in Peptide Mapping Workflows

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Overview

Peptide mapping is a common method for protein characterization. The general workflow includes the isolation of a protein, followed by in-solution digest using a serine protease to yield peptides, which are subsequently analyzed by LC and/or MS techniques. Because of its specificity and the general size of peptides generated, trypsin is most commonly used. To optimize sequence coverage, the protein must be denatured prior to trypsin digestion. In this application note, we investigate the differences in results for sequence coverage and overall chromatographic performance using two commonly used chaotropic agents for sample denaturation - urea and guanidine HCl.

In general, the guanidine digested samples yielded a higher number of unique peptides generated; 287 unique peptides were observed with guanidine digested samples when compared to 237 with urea. This can even visually be observed in the Total Ion Chromatogram (TIC) comparison in **Figure 1**. Additionally, guanidine digested samples resulted in a higher sequence coverage for heavy chain. Based on these results, it is clear why guanidine is preferred over urea in most peptide mapping workflows.

Analysis of specific peptide sequences demonstrates the advantage of guanidine as a denaturant. A longer peptide, DYFP, is observed with a guanidine reduction, but not when using urea (**Figure 2**). Moreover, smaller peptides such as VSNK and TISK, are yielded at a higher efficiency with the guanidine protocol (**Figure 3**).

In summary, peptide mapping protocols vary dependent on the protein and desired sequence, among other factors. These results show the superior chaotrope of guanidine HCl compared to another common chaotrope, urea.

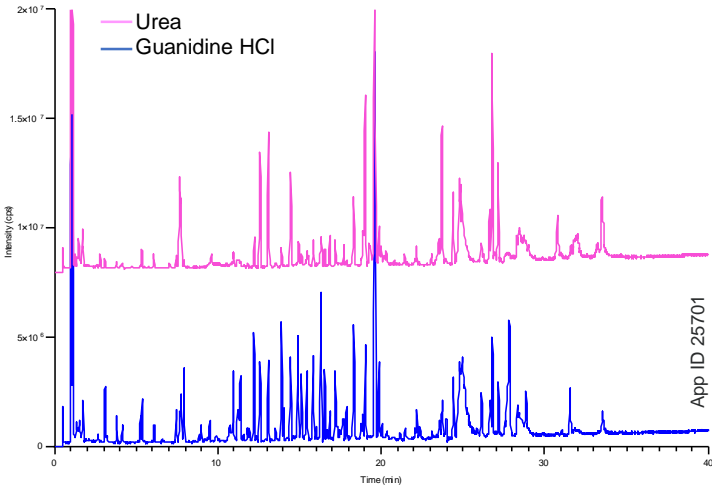
Digestion Procedure:

Step	Details
Denaturation	To sample, add 1:1 (v:v) of 5 M Guanidine HCl:Protein or 1:1 (v:v) 8 M Urea:Protein
Reduction	1:10 (v:v) 200 mM DTT:Protein Incubate at 57 °C for 30 min, shaking at 1000 rpm
Alkylation	1:2 (v:v) 400 mM iodoacetamide (IAM): DTT Incubate in the dark 45 min Quench, 1:2 (v:v) 200 mM DTT: IAM
Buffer Exchange	100 mM Ammonium Bicarbonate, overnight
Digestion	1:20 Trypsin:Sample (w:w) Incubate 37 °C for 6 h, shaking at 1000 rpm
Reaction Quench	Formic acid SpeedVac to dryness, resuspend in mobile phase prior to analysis

LC Conditions

Column: bioZen™ 2.6 µm Peptide XB-C18
Dimension: 150 x 2.1 mm
Part No.: [00F-4768-AN](#)
Recommended Guard: SecurityGuard™ ULTRA
Guard Cartridge Part No.: [AJ0-9806](#)
Guard Holder Part No.: [AJ0-9000](#)
Mobile Phase: A: 0.1 % Formic Acid in Water
 B: 0.1 % Formic Acid in Acetonitrile
Flow Rate: 0.3 mL/min
Gradient: 1-50% B in 50 minutes
Temperature: 40 °C
Detector: Q-TOF (SCIEX® X500B)
Sample: Tryptic digest, Trastuzumab

Figure 1. Urea vs Guanidine HCl Denatured Trastuzumab



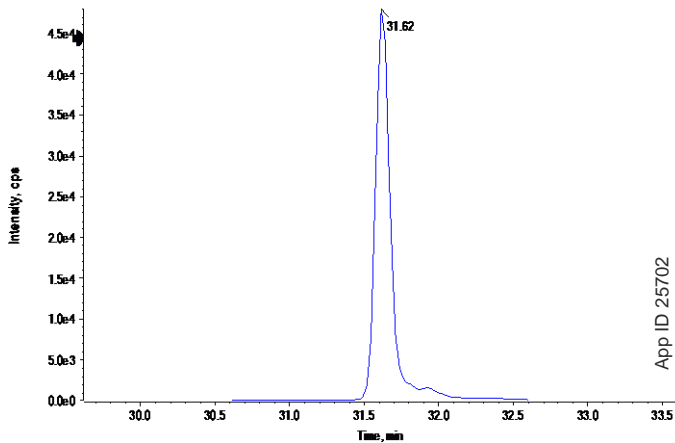
Comparison of Sequence Coverage

Urea Denatured Trastuzumab:

Heavy Chain Sequence Coverage 72.1%

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
 QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSK
 NTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT
 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
 FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPPKSCDKTH
 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
 VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 2. XIC of DYFP Peptide

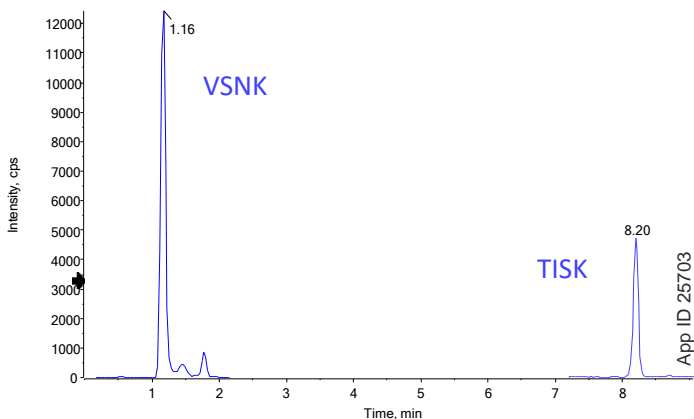


Guanidine Denatured Trastuzumab:

Heavy Chain Sequence Coverage 92.9%

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
 QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSK
 NTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT
 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
 FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPPKSCDKTH
 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
 VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK


Figure 3. XICs of VSNK and TISK Peptides



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